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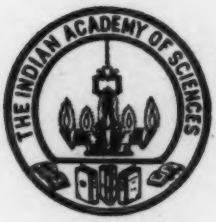

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PHÆOTRICHOCONIS, A NEW GENUS OF THE DEMATIACEÆ

By C. V. Subramanian, F.A.Sc.

Received June 8, 1956

Trichoconis crotalariæ, which forms the subject of this paper, was described by Salam and Rao (1954) from a collection made in Hyderabad Deccan on leaves and pods of *Crotalaria verrucosa* L. They described the fungus as follows: "Spots irregular, white, mycelium well developed, hyphæ 3·6–6 μ thick, branched, septate when mature at intervals of 18–36 μ..... Conidiophores not sharply distinguishable from hyphæ, erect, septate, with swollen apex, 52–156 μ long, 3·6–6 μ broad, bearing one conidium each; conidia of a characteristic shape, elongate, fusiform with a long appendage at the apex, attached by their broad end, and having a dark scar showing the point of attachment, 5–8-septate, dark brown, not constricted at the septa, thick-walled, with granular contents; the second or third cell from the base larger than the rest, conidia 46·8–95 $\times$ 10·4–18·2 μ; appendage hyaline, non-septate, and slightly curved, longer than the body of the conidium, 49·4–127·5 μ....." (Salam and Rao, 1954, p. 190).

The genus *Trichoconis* was established in 1909 by Clements to accommodate *Piricularia caudata* App. and Str., which was considered by him to be distinct from the genus *Piricularia* Sacc., mainly because *P. caudata* had, as its name indicates, caudate conidia (Saccardo, 1931, p. 721). Indeed, this fungus was compiled under *Appelia* Sacc., characterised as "conidia apice setigera" by Saccardo as a subgenus of *Piricularia* Sacc. (Saccardo, 1906, p. 545). A description of this fungus, from Saccardo (1906), is given below: "Maculis atro-brunneis, non-distincte limitatis; cæspitulis albidis, effusis; mycelium parum evoluto, 3–4 μ cr., hyalino; conidiophoris erectis 100–150 μ long. parce septatis, filiformibus, apice monosporis; conidiis breve fusoideis, rectiusculis, 2–4-septatis, non-constrictis, hyalinis, apice in setulam filiformem rectam v. curvulam subæquilongam productis, 36–45 $\times$ 9–12 μ, setula 35–45 μ long." It will be clear from the above description that *Trichoconis caudata* (App. and Str.) Clem. ($\equiv$ *Piricularia caudata* App. and Str.) which is the type species of the genus *Trichoconis*, is a moniliaceous hyphomycete with hyaline mycelium and hyaline conidia.

Trichoconis crotalariæ, on the other hand, is distinctly dematiaceous, having dark-coloured mycelium and producing dark brown conidia, as

described by its authors and as confirmed by a study of the type material very kindly made available to me by the authors. It is, therefore, not congeneric with the moniliaceous *T. caudata*. I know of no other genus of the Dematiaceæ-Phragmosporæ in which it can be placed and I am, therefore, proposing a new genus for it. The generic name being proposed, *viz.*, *Phæotrichoconis*, indicates that it is Dematiaceous, but otherwise resembles *Trichoconis*.

Phæotrichoconis Subramanian gen. nov.

Pertinet ad Fungos Imperfectos, ad Dematiaceas, atque ad Phragmosporas.

Hyphæ repentes brunneæ, ramosæ, septatæ. Conidiophori haud clare separabilis ad hyphis, erecti, septati, apice tumescentes, unum conidium ferentet singuli. Conidia acrogena, brunnea, elongata, fusiformia, appendice longa ad apicem ornata, sæpe transverse septata.

Fungus Imperfectus, Dematiaceæ, Phragmosporæ.

Repent hyphæ brown, branched, septate. Conidiophores not sharply distinguished from hyphæ, erect, septate, with swollen apex, each bearing apically and singly one conidium. Conidia acrogenous, brown, elongate, fusiform, with a long appendage at the apex, many times transversely septate.

Type species

Phæotrichoconis crotalariæ (Salam and Rao) Subramanian comb. nov.

Basonym.—Trichoconis crotalariæ Salam and Rao, 1954, in *J. Indian bot. Soc.*, **33**, 191, ic.

Type.—On leaves and pods of *Crotalaria verrucosa* L., Agricultural College Farm, Osmania University, Hyderabad-Deccan, 22–4–1953, coll. M. A. Salam and P. N. Rao; culture deposited at the Commonwealth Mycological Institute, Kew (Herb. IMI 52821).

I am deeply indebted to Dr. M. A. Salam and Shri P. N. Rao of the Botany Department of the Osmania University for making available type material of *Trichoconis crotalariæ*.

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——— .. *Ibid.*, 1931, **25**, 1093, lithoprinted by Edwards Brothers, Michigan, 1944.

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CHEMICAL STUDIES ON INDIAN SEAWEEDS

I. Mineral Constituents*

By V. Krishna Pillai

(Central Marine Fisheries Research Station, Mandapam Camp)

Received May 4, 1956

(Communicated by Dr. N. K. Panikkar, F.A.SC.)

Introduction

There is practically no reference which gives a complete account of the mineral constituents of the commercial seaweeds and their relationship with the organic constituents or on the ionic exchanges taking place between the algæ and the surrounding water. The early attempts to utilize seaweeds for the manufacture of potash and iodine provide some data on the amounts of these elements and their seasonal variations in particular species. Work on British seaweeds has shown (Chapman, 1950) that the changes in the iodine content are nearly directly proportional to the potash and that the amount of these nutrients in the living plant varies from month to month.

Webb (1937) carried out a spectrographic analysis of three algæ (*Ulva lactuca*, *Fucus serratus* and *Saccorhiza bulbosa*) and obtained values for sodium, potassium, calcium, magnesium, strontium, barium, boron, molybdenum, aluminium, manganese, iron, copper, lead, etc. Cornec (1919) detected silver, arsenic, cobalt, copper, manganese, zinc, bismuth, tin, barium, molybdenum, gold, antimony, titanium and uranium in marine plants. The analysis was done by spectroscopic methods and was purely qualitative. The species analysed have not been given. Jones (1922) estimated the arsenic content of eleven species of British seaweeds and obtained a value of 125 mg./kg. in *Laminaria digitata*. Later Williams and Whetstone (1940) also made studies on the distribution of arsenic in marine algæ. Meulan (1932) studied the molybdenum content of several species of algæ and reports a figure of 0·16 mg./kg. dry weight. Igelsrud, Thompson and Zwicker (1938) give the range of boron content in a few seaweeds as between 4·2 and 14·9 mg./kg. dry material. Even some of the rare earth elements have been detected in *Laminaria cloustoni* by Servigne and Tchakirian (1939), who found about 5 mg./kg. of Pr, Nd and Sm. Accumulation of radium in algæ has been observed by Wiesner (1938) analysing more than

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20 species of algæ of both fresh- and salt-water origin. Oy (1940) discusses in detail the physiological importance of iron, copper, manganese and boron and estimates these elements in several species of seaweeds, *viz.*, *Laminaria* spp., *Fucus serratus* and *Fucus vesiculosus*.

An analysis of a dry seaweed meal has been reported by Beharrell (1942), and in 1941 Wilson and Fieldes estimated spectrographically eighteen of the minor elements in *Macrocystis pyrifera*. Black and Mitchell (1952) in a detailed study have reported the cobalt, nickel, molybdenum, iron, lead, tin, zinc, manganese, barium, copper, chromium, titanium, rubidium, silver, vanadium, lithium and strontium contents of the following species of brown algæ: *Pelvetia canaliculata*, *Ascophyllum nodusum*, *Fucus serratus*, *Laminaria digitata* and *Laminaria cloustoni*; in certain cases the fronds and stipes have been separately analysed. In this work an attempt has been made to study the seasonal variations in the trace element contents of particular species; and the data obtained show positive variation in the concentration of the trace elements investigated. The authors also have correlated the trace element content of the surrounding sea-water with that of the seaweeds and have established that trace elements accumulate to a very great extent in the seaweeds.

The foregoing review bears out that the chief concept in the existing literature on the subject of the chemical composition of seaweeds is variation in the individual species with the condition of the surrounding water, with depth and with seasons. But the entire work referring as it does to the seaweeds of temperate regions leaves the subject untouched so far as the seaweeds of the tropical waters are concerned.

EXPERIMENTAL

In the present communication a preliminary account is given on the seasonal changes in the chemical composition of 11 species of seaweeds common to the Indian coast. The species studied are:

1.	*Chætomorpha linum* Kutz.	Chlorophyceæ
2.	*Enteromorpha intestinalis* Link.	
3.	*Gracilaria lichenoides* (L.) Harv.	Rhodophyceæ
4.	*Chondria dasyphylla* (Woodw.) Ag.	
5.	*Acanthophora spicifera* (Vahl.) Boerg.	
6.	*Laurencia papillosa* (Forssk.) Grev.	
7.	*Hypnea musciformis* (Wulf.) Lamour.	
8.	*Sarconema filiforme* (Sond.) Kylin.	
9.	*Sarconema furcellatum* Zan.	

10. *Rosenvingia intricata* (J. Ag.) Boerg.
11. *Padina australis* Hauck. } Phæophyceæ

Among these *Chætomorpha linum, Gracilaria lichenoides, Hypnea musciformis* and *Rosenvingia intricata* form a floating association while the others are attached forms. The samples were collected from Palk Bay near Mandapam. The littoral area is shallow and is fringed by a coral reef, the dead coral masses and fragments providing a firm substrate for the attachment of the algæ. The Bay being sheltered permits a floating association as well. The first collection of the algæ was made in April 1952, and subsequently once in a month until March 1953. By selecting the above species for investigation it was felt that the characteristic differences between different species in the same group could be studied. Since almost all the seaweeds (both floating as well as attached forms) were available throughout the year the changes in their composition during different stages of growth could also be studied.

The fresh samples after removal of sand and calcareous particles were thoroughly washed in fresh-water and left for drying in the sun. The atmospheric temperature was sufficiently high for proper drying of the samples. A few species like *E. intestinalis, G. lichenoides, L. papillosa* and *P. australis* were analysed in the fresh condition with a view to study the changes in the water-soluble minerals in them.

Methods of Analysis

For the estimation of water-soluble constituents known quantities of the seaweed, after superficial washing in distilled water, is ground to a paste with acid-washed sand adding distilled water to requirement. The paste and the washings are centrifuged and the clear extract taken. The process is repeated three times and the combined extracts are made upto a known volume.

Estimation of total minerals.—For each estimation the ash obtained from 2 grams of the dry seaweed are used in the case of the major elements sodium, potassium, calcium, magnesium, and chlorine. As against this about 10 to 20 grams of the seaweed will be required for the estimation of trace elements. The methods employed in the analysis of the above constituents are the same as reported in an earlier communication (Krishna Pillai, 1955).

With a view to understand the form in which the two elements Mn and B exist in the different seaweeds, a series of analysis were conducted on representative collections of young and mature specimens of all the 11 species mentioned above.

The results of analysis on the water-soluble constituents of the 4 species of seaweeds are tabulated in Tables I to VI. The moisture, ash, sodium, potassium, calcium, magnesium and chlorine of the different species collected during the various months are represented in Graphs 1 to 11. The seasonal variations in the trace element contents of the seaweeds are given in Tables VII to XIV.

TABLE I

Water-Soluble Minerals in Seaweeds Collected during June 1952

Values expressed as percentage on wet weight. (Figures in brackets are values on dry weight as calculated from wet weight)

Name of Species	Na	K	Ca	Mg	Cl
A. spicifera	0·03	0·07	0·04	0·03	0·26
	(0·32)	(0·18)	(0·42)	(0·38)	(3·06)
L. papillosa	0·05	0·08	0·04	0·04	0·26
	(0·42)	(0·61)	(0·29)	(0·31)	(2·10)
S. furcellatum	0·08	0·05	0·02	0·02	0·28
	(0·56)	(0·31)	(0·16)	(0·15)	(1·80)
P. australis	0·12	0·14	0·05	0·08	0·22
	(0·78)	(0·93)	(0·32)	(0·50)	(1·40)

TABLE II

Water-Soluble Minerals in Seaweeds Collected during August 1952

Values expressed as percentage on wet weight. (Figures in brackets are values on dry weight as calculated from wet weight)

Name of Species	Na	K	Ca	Mg	Cl
G. lichenoides	0·03	0·11	0·04	0·04	0·42
	(0·20)	(0·81)	(0·28)	(0·28)	(2·80)
P. australis	0·14	0·14	0·12	0·07	0·40
	(0·75)	(0·76)	(0·65)	(0·41)	(2·20)

TABLE III

Water-Soluble Minerals in Seaweeds Collected during September 1952

Values expressed as percentage on wet weight. (Figures in brackets are values on dry weight as calculated from wet weight)

Name of Species	Na	K	Ca	Mg	Cl
L. papillosa		0·05 (0·41)	0·04 (0·31)	..	0·15 (1·30)
S. furcellatum		0·05 (0·40)	0·06 (0·51)	..	0·22 (1·81)

TABLE IV

Water-Soluble Minerals in Seaweeds Collected during October 1952

Values expressed as percentage on wet weight. (Figures in brackets are values on dry weight as calculated from wet weight)

Name of Species	Na	K	Ca	Mg	Cl
G. lichenoides	0·04	0·10 (0·75)	0·04 (0·30)	0·02 (0·16)	0·45 (3·70)
L. papillosa ..	0·10 (0·88)	0·09 (0·82)	0·07 (0·61)	0·03 (0·29)	0·25 (2·00)
P. australis	0·21 (1·28)	0·15 (0·90)	0·09 (0·50)	..	0·39 (2·40)

TABLE V

Water-Soluble Minerals in Seaweeds Collected during December 1952

Values expressed as percentage on wet weight. (Figures in brackets are values on dry weight as calculated from wet weight)

Name of Species	Na	K	Ca	Mg	Cl
E. intestinalis	0·04 (0·58)	0·05 (0·63)	0·02 (0·28)	..	0·08 (1·25)
G. lichenoides	0·02 (0·23)	0·21 (2·01)	0·04 (0·40)	..	0·20 (2·00)
L. papillosa	0·15 (1·16)	0·11 (0·70)	0·04 (0·31)	..	0·32 (2·40)

TABLE VI

Water-Soluble Minerals in Seaweeds Collected during February 1953

Values expressed as percentage on wet weight. (Figures in brackets are values on dry weight as calculated from wet weight)

Name of Species	Na	K	Ca	Mg	Cl
G. lichenoides	0·03	0·18	0·02	..	0·55
	(0·20)	(1·25)	(0·11)		(3·84)
E. intestinalis	0·06	0·05	0·04	0·03	0·14
	(0·73)	(0·61)	(0·51)	(0·41)	(1·70)

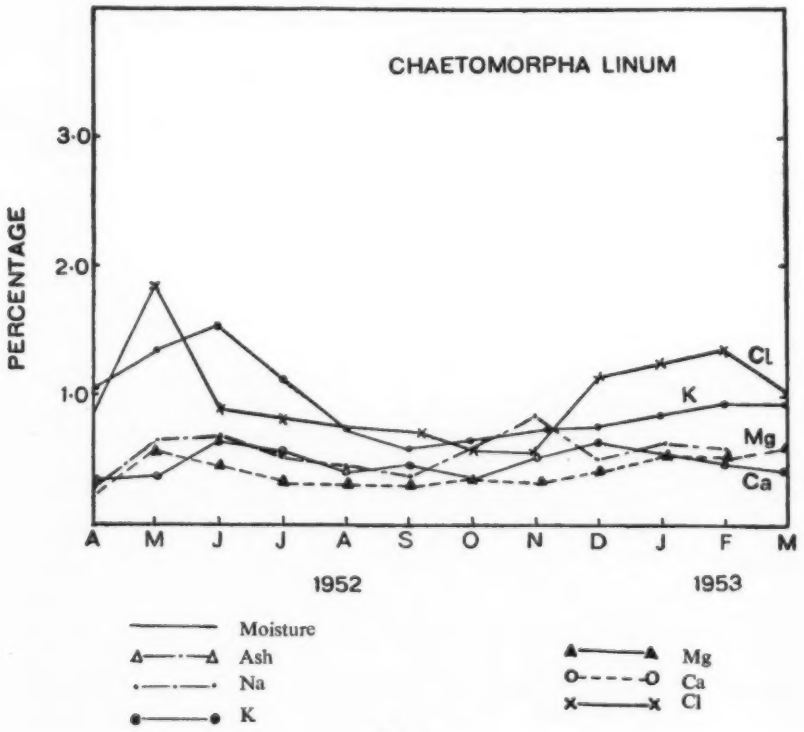


FIG. 1

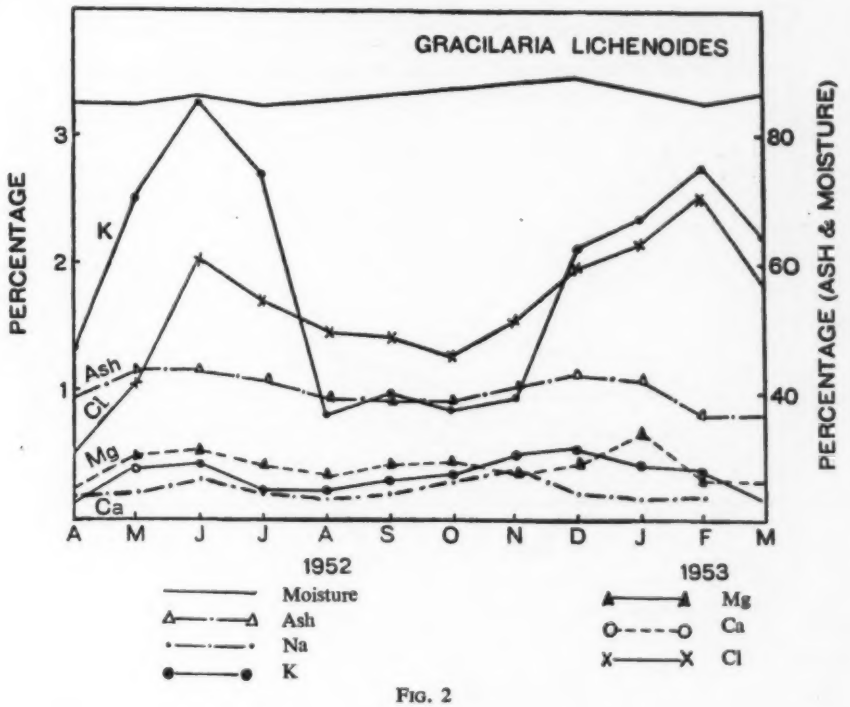


FIG. 2

TABLE VII

Trace Elements in Seaweeds Collected during April 1952

(Values given as milligram per kilogram of air-dried material)

Name of Species	Fe	Cu	Mn	B	Mo	I	Zn
C. linum ..	42·0	5·0	46·0	1·0	trace	32·0	13·0
G. lichenoides ..	70·0	3·0	112·0	2·8	0·08	115·0	26·0
C. dasyphylla ..	126·0	2·6	73·0	trace	trace	74·0	20·0
E. intestinalis ..	38·5	trace	38·0	1·6	0·06	34·0	10·0
A. spicifera ..	140·0	trace	40·0	4·3	trace	79·0	25·0
L. papillosa ..	160·0	1·8	91·0	1·0	trace	39·5	24·0
H. musciformis	196·0	3·2	69·0	4·0	0·10	98·7	43·0
S. filiforme ..	83·0	2·4	95·0	3·7	0·10	86·4	24·0
S. furcellatum ..	76·0	2·0	56·0	1·8	0·07	99·0	32·0
R. intricata ..	129·0	4·0	240·0	1·8	0·06	123·0	15·0
P. australis ..	237·0	1·4	46·0	2·0	0·10	79·0	28·0

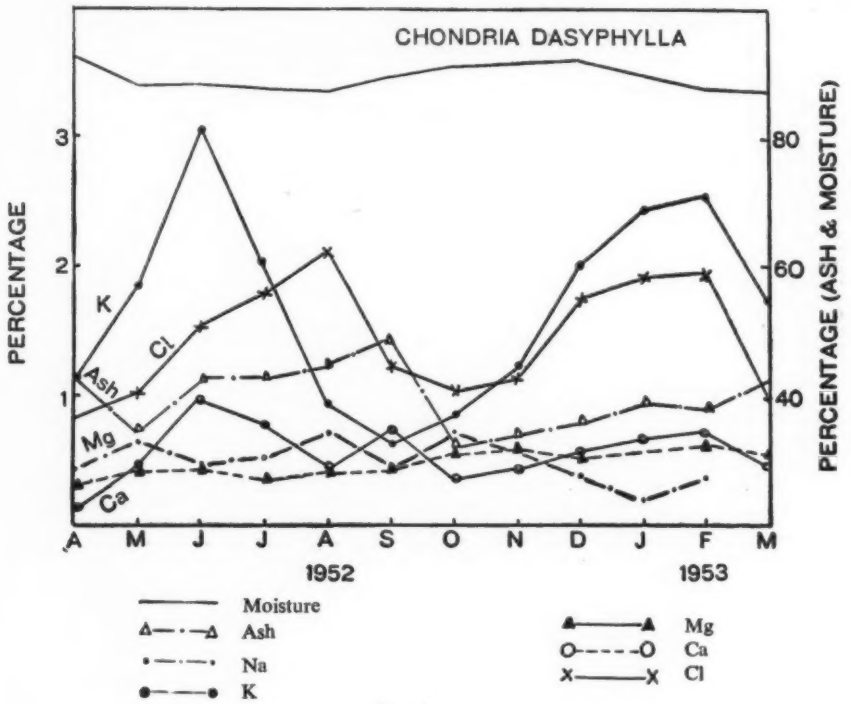


FIG. 3

TABLE VIII

Trace Elements in Seaweeds Collected during May 1952

(Values given as milligram per kilogram of air-dried material)

Name of Species	Fe	Cu	Mn	B	Mo	I	Zn
C. linum ..	112·0	3·8	..	trace	trace	64·0	18·0
G. lichenoides ..	..	5·0	trace	1·8	0·08	123·0	19·0
C. dasyphylla ..	144·2	trace	87·0	1·6	trace	96·0	16·0
E. intestinalis ..	126·0	1·5	30·0	1·2	trace	58·0	18·0
A. spicifera ..	128·8	10·0	trace	3·1	trace	88·0	15·0
L. papillosa ..	190·4	5·0	trace	3·6	..	60·0	15·0
H. musciformis	210·0	9·0	180·0	3·8	0·08	60·0	26·0
S. filiforme ..	65·0	6·5	63·0	1·9	0·08	96·0	20·0
S. furcellatum ..	70·0	30·0	200·0	2·0	trace	74·0	28·0
R. intricata ..	119·0	5·0	575·0	7·0	0·05	264·0	12·0
P. australis ..	259·0	trace	195·0	11·0	0 08	246·0	28·0

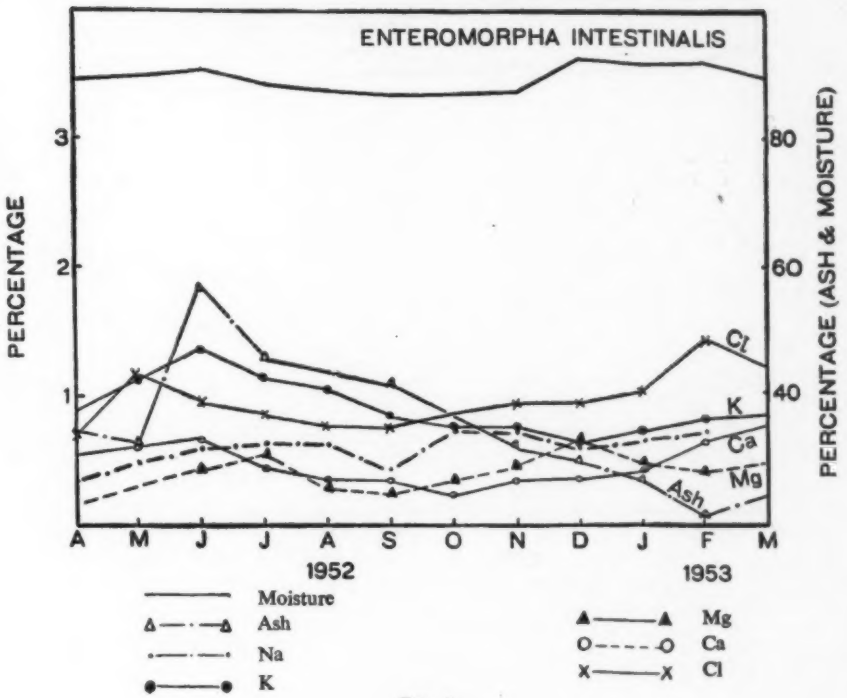


FIG. 4

TABLE IX

Trace Elements in Seaweeds Collected during June 1952

(Values given as milligram per kilogram of air-dried material)

Name of Species	Fe	Cu	Mn	B	Mo	I	Zn
C. linum ..	100·8	5·0	35·0	2·7	0·05	72·0	16·0
G. lichenoides ..	79·8	4·6	163·0	10·4	0·20	108·0	59·0
C. dasyphylla ..	112·0	6·0	140·0	8·0	0·10	72·0	37·0
E. intestinalis ..	86·4	2·2	80·0	4·5	0·10	41·0	21·0
A. spicifera ..	87·5	3·5	20·0	2·2	0·06	76·0	7·0
L. papillosa ..	129·5	Nil	85·0	4·6	0·06	24·0	14·0
H. musciformis ..	185·2	5·0	135·0	8·0	0·10	12·0	28·0
S. filiforme ..	85·6	2·0	187·0	7·3	0·10	56·0	20·0
S. furcellatum ..	80·5	1·8	..	9·4	0·05	160·0	58·0
R. intricata ..	136·5	0·8	181·0	7·4	0·06	30·0	26·0
P. australis ..	251·6	11·2	208·0	11·0	0·08	186·0	40·0

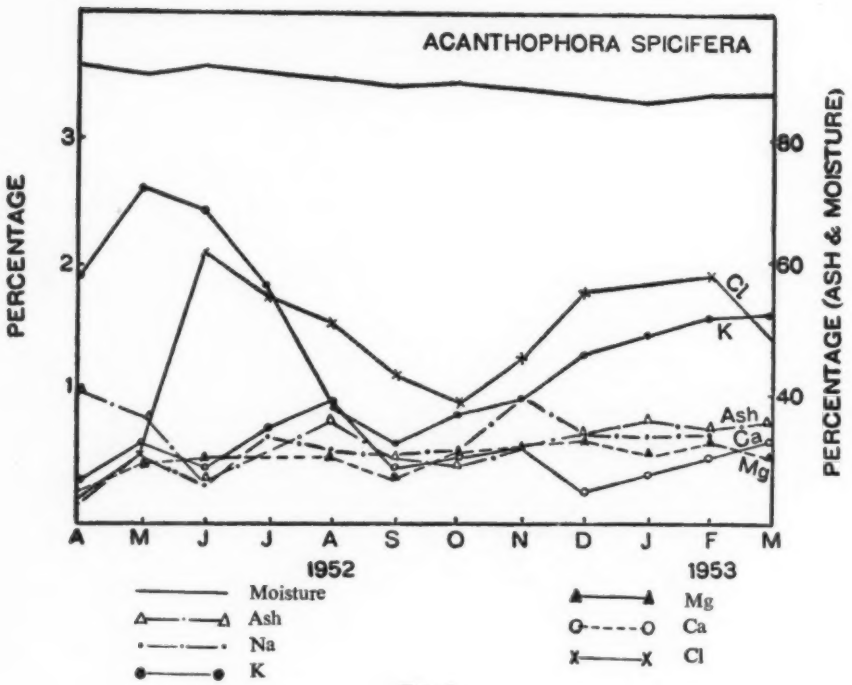


FIG. 5

TABLE X

Trace Elements in Seaweeds Collected during August 1952

(Values given as milligram per kilogram of air-dried material)

Name of Species	Fe	Cu	Mn	B	Mo	I	Zn
C. linum ..	198·0	4·0	210·0	4·4	trace	..	..
G. lichenoides ..	70·0	10·0	550·0	12·8	0·24	119·0	65·0
C. dasyphylla ..	186·0	6·0	155·0	8·5	0·10	90·0	34·0
E. intestinalis ..	103·0	..	130·0	6·0	0·08	..	28·0
A. spicifera ..	105·0	12·0	85·0	2·8	0·06	90·0	17·0
L. papillosa ..	140·0	3·8	240·0	4·4	0·05	trace	11·0
H. musciformis	172·0	Nil	195·0	7·5	0·09	trace	24·0
S. filiforme ..	103·0	trace	160·0	..	0·12	..	33·0
S. furcellatum ..	63·0	Nil	390·0	8·6	0·08	357·0	65·0
R. intricata ..	214·0	1·1	140·0	..	0·06	32·0	23·0
P. australis ..	315·0	2·5	450·0	9·0	0·10	212·0	37·0

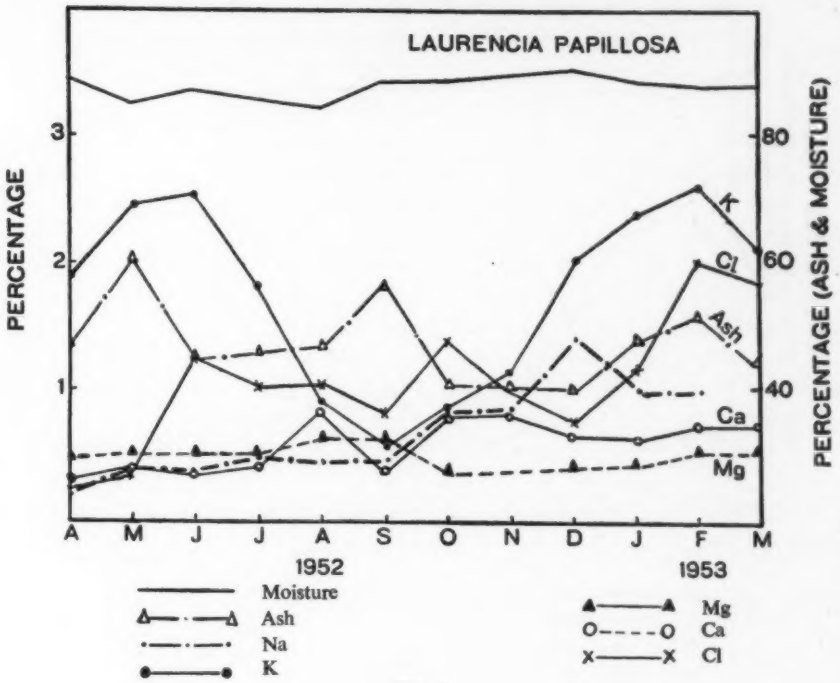


FIG. 6

TABLE XI

Trace Elements in Seaweeds Collected during September 1952

(Values given as milligram per kilogram of air-dried material)

Name of Species	Fe	Cu	Mn	B	Mo	I	Zn
C. linum ..	217·0	4·0	385·0	1·2	0·08	Nil	30·0
G. lichenoides ..	280·0	..	149·0	..	0·10	63·0	60·0
C. dasyphylla ..	248·5	1·0	82·5	1·0	0·08	..	68·0
E. intestinalis ..	78·0	..	64·0	..	..	15·0	44·0
A. spicifera ..	210·0	3·8	60·5	Nil	trace	Nil	52·0
L. papillosa ..	140·0	2·5	104·5	1·6	trace	Nil	trace
H. musciformis	186·5	trace	173·1	3·0	..	14·0	43·0
S. filiforme ..	143·0	4·0	..	..	0·06	..	37·0
S. furcellatum ..	35·0	2·4	85·0	2·6	0·10	23·0	54·0
R. intricata ..	208·0	trace	165·0	2·6	trace	164·0	20·0
P. australis ..	372·0	trace	140·0	3·0	trace	153·0	29·0

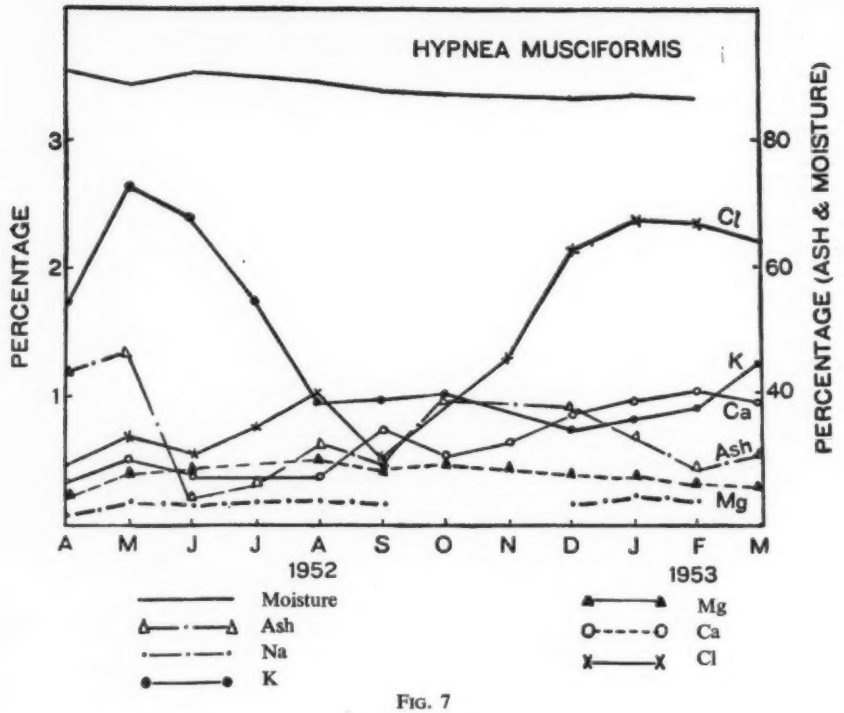


FIG. 7

TABLE XII

Trace Elements in Seaweeds Collected during October 1952

(Values given as milligram per kilogram of air-dried material)

Name of Species	Fe	Cu	Mn	B	Mo	I	Zn
C. linum ..	88·0	1·8	..	..	trace	14·0	..
G. lichenoides ..	196·0	5·0	171·0	4·0	0·12	125·0	65·0
C. dasyphylla ..	292·4	7·5	82·5	2·5	0·09	60·0	..
E. intestinalis ..	140·0	trace	87·4	..	..	32·0	..
A. spicifera ..	280·0	12·0	51·3	2·0	trace	20·0	70·0
L. papillosa ..	378·0	3·5	93·5	3·5	Nil	18·0	50·0
H. musciformis	280·0	5·0	104·0	6·5	0·05	24·0	50·0
S. filiforme ..	196·0	2·4	65·4	3·9	trace	56·0	..
S. furcellatum ..	140·0	2·6	73·6	6·2	0·10	116·0	30·0
R. intricata ..	224·0	trace	115·5	4·3	trace	263·0	..
P. australis ..	504·0	trace	123·4	4·8	Nil	275·0	..

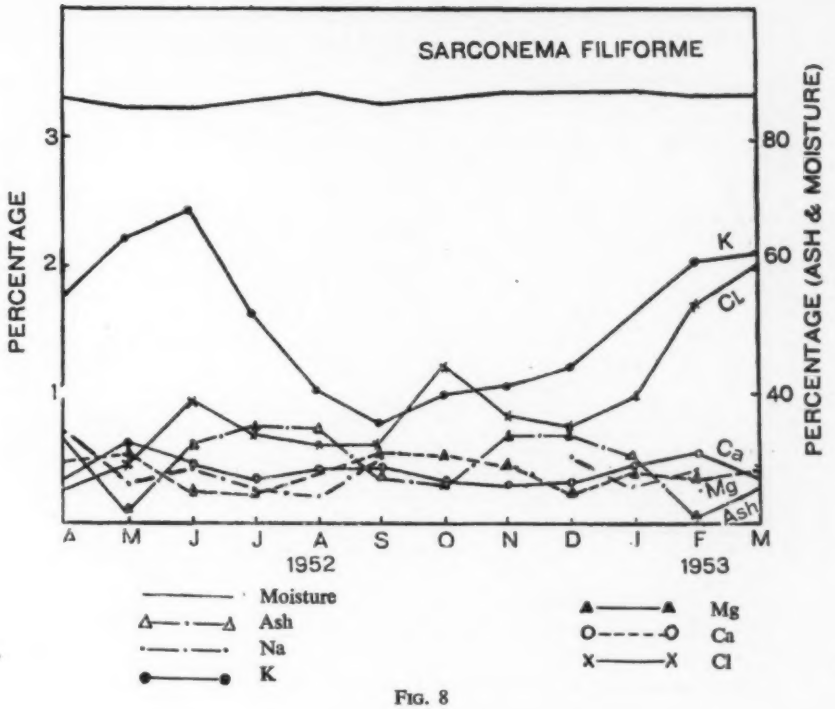


FIG. 8

TABLE XIII

Trace Elements in Seaweeds Collected during December 1952

(Values given as milligram per kilogram of air-dried material)

Name of Species	Fe	Cu	Mn	B	Mo	I	Zn
C. linum ..	90·0	..	..	3·0	0·10	10·0	29·0
G. lichenoides ..	112·0	5·0	200·0	14·3	0·12	175·0	76·0
C. dasyphylla ..	350·0	6·0	175·0	2·4	0·16	98·0	46·0
E. intestinalis ..	110·0	2·5	55·0	..	0·05	30·0	30·0
A. spicifera ..	126·0	1·5	40·0	2·6	trace	29·0	14·0
L. papillosa ..	164·0	3·8	97·5	3·0	0·05	40·0	26·0
H. musciformis	112·0	2·5	55·0	7·0	0·05	84·0	46·0
S. filiforme ..	70·0	2·5	93·0	4·8	0·10	107·0	53·0
S. furcellatum ..	..	..	55·0	2·3	0·09	212·0	40·0
R. intricata ..	165·0	Nil	76·0	5·0	trace	449·0	32·0
P. australis ..	273·0	Nil	136·0	3·4	0·10	500·0	30·0

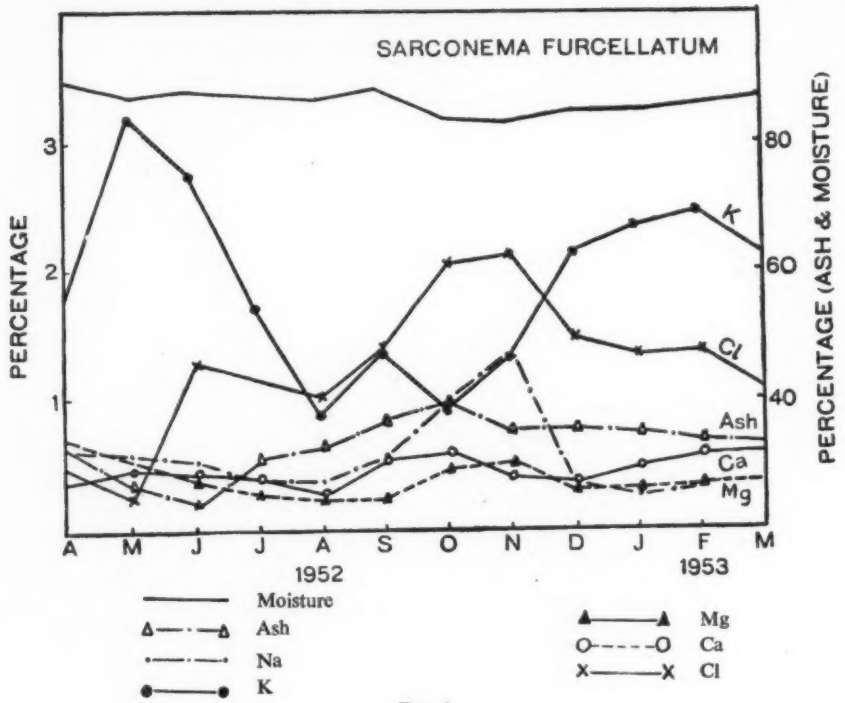


FIG. 9

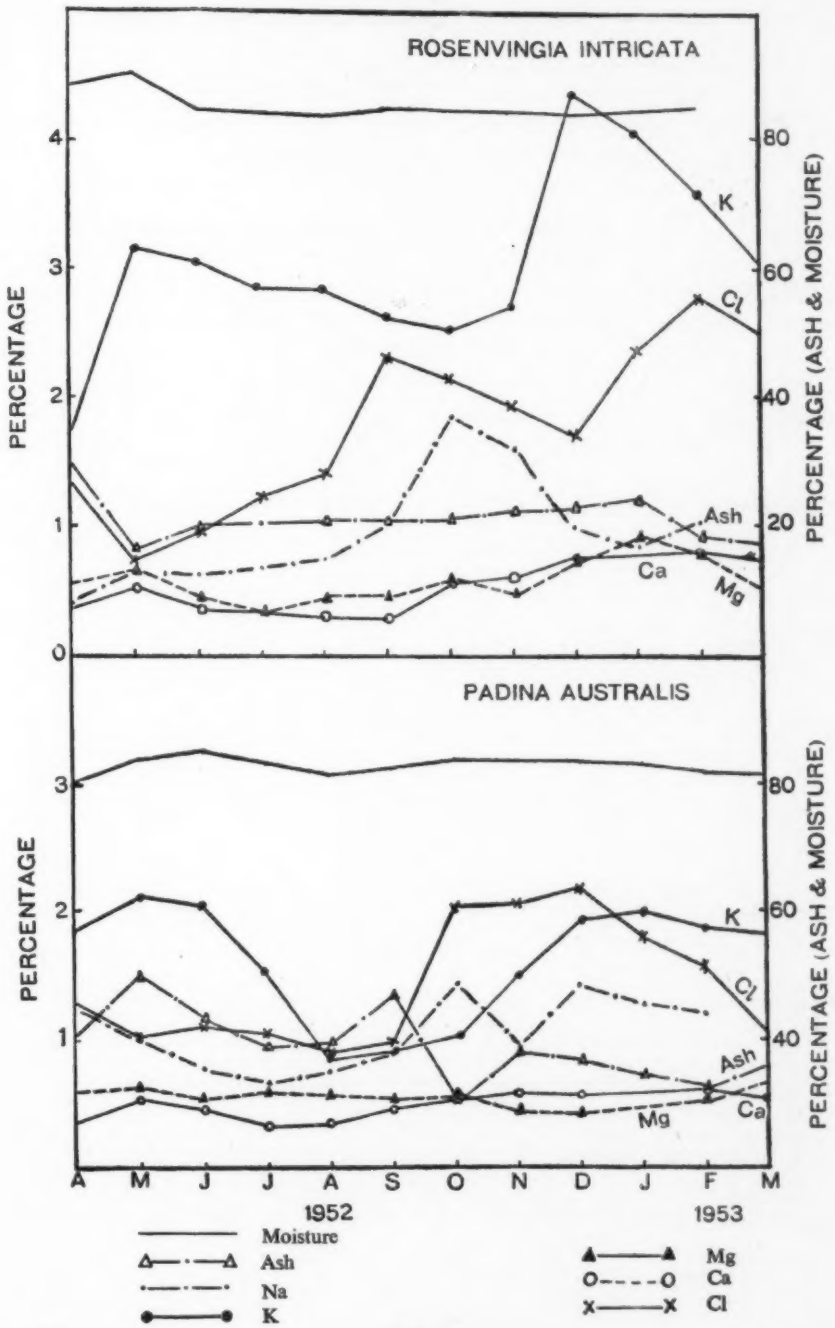
TABLE XIV

Trace Elements in Seaweeds Collected during February 1953

(Values given as milligram per kilogram of air-dried material)

Name of species	Fe	Cu	Mn	B	Mo	I	Zn
C. linum ..	124·0	2·3	275·0	1·6	0·10	24·0	18·0
G. lichenoides ..	189·0	5·6	168·0	6·4	0·16	208·0	83·0
C. dasyphylla ..	308·0	9·0	36·5	4·0	0·16	96·0	48·0
E. intestinalis ..	86·0	1·3	58·0	3·0	0·08	29·0	14·0
A. spicifera ..	..	trace	55·0	1·8	0·05	63·0	46·0
L. papillosa ..	140·0	trace	137·5	2·0	0·05	137·0	55·0
H. musciformis	..	2·7	..	5·0	0·05	100·0	80·0
S. filiforme ..	140·0	2·0	142·4	Nil	0·10	98·0	64·0
S. furcellatum ..	..	..	..	..	0·12	198·0	57·0
R. intricata ..	210·0	..	105·0	3·8	0·08	430·0	31·0
P. australis ..	231·0	..	165·0	1·9	0·10	470·0	44·0

FIG. 10



B2

FIG. 11

The total, as well as cold water and hot water-soluble manganese and boron contents of the seaweeds are given in Tables XV and XVI respectively.

TABLE XV

Partition of Manganese in Seaweeds during Different Stages of Growth

(Figures in brackets are percentages in total Mn)

Name of Species	Mature Specimen			Young Specimen		
	Total mg./kg.	Cold-water-soluble Mn mg./kg.	Hot-water-soluble Mn mg./kg.	Total mg./kg.	Cold-water-soluble mg./kg.	Hot-water-soluble mg./kg.
G. lichenoides ..	550·0	170·0 (31)	200·0 (36·4)	200·0	120·0 (60)	120·0 (60)
C. dasyphylla ..	155·0	65·0 (42)	75·0 (48)	175·0	90·0 (51)	95·0 (54)
E. intestinalis ..	130·0	..	..	55·0	..	..
A. spicifera ..	85·0	15·0 (18)	15·0 (18)	40·0	20·0 (50)	20·0 (50)
L. papillosa ..	140·0	35·0 (25)	40·0 (28·5)	97·5	42·5 (48)	46·0 (49)
H. musciformis ..	195·0	40·0 (21)	50·0 (26)	100·0	65·0 (65)	70·0 (70)
S. furcellatum ..	390·0	40·0 (10)	60·0 (15)	55·0	15·0 (27)	20·0 (36)
P. australis ..	450·0	150·0 (33)	150·0 (33)	113·4	55·4 (48)	60·4 (54)

TABLE XVI

Partition of B in Seaweeds

(Figures in brackets indicate percentages on total B content)

Name of Species	Mature Specimens			Young Specimens		
	Total mg./kg.	Cold-water-soluble mg./kg.	Hot-water-soluble mg./kg.	Total mg./kg.	Cold-water-soluble mg./kg.	Hot-water-soluble mg./kg.
G. lichenoides ..	12·8	0·90 (7·0)	0·94 (7·3)	4·0	0·38 (9·5)	0·42 (10·5)
C. dasyphylla ..	8·5	0·68 (8·0)	0·67 (7·9)	2·5	0·26 (10·4)	0·24 (9·6)
E. intestinalis ..	6·0	0·35 (5·8)	0·41 (6·8)	..	..	..
A. spicifera ..	2·8	0·24 (8·5)	0·28 (10·0)	2·0	0·21 (10·5)	0·21 (10·5)
L. papillosa ..	4·4	0·45 (10·2)	0·44 (10·0)	3·5	0·30 (8·6)	0·33 (9·4)
H. musciformis ..	7·5	0·50 (6·7)	0·55 (7·5)	6·5	0·70 (10·8)	0·70 (10·8)
S. furcellatum ..	8·6	0·60 (7·0)	0·62 (7·2)	4·3	0·39 (9·0)	0·42 (9·8)
P. australis ..	9·0	0·94 (10·4)	0·95 (10·6)	4·8	0·46 (9·6)	0·46 (10·0)

DISCUSSION OF RESULTS

From an examination of the fresh seaweed samples immediately after bringing them to the laboratory it was observed that those collected between the months of March and July were fully mature and fruiting. Collections

made after August were a mixture of fruiting and young specimens, the proportion of young plants increasing until December when the collections were found to contain only non-fruiting young plants.

Black (1948) has shown beyond doubt that marked seasonal variations in chemical composition occur mainly in the fronds, where most photosynthesis takes place, while seasonal variations in the stipes are only very little. It is not logical, he has pointed out, to expect vast changes in the chemical composition of stipes which being perennial are unlike the annually formed fronds. In the present study four of the eleven species taken up are members of a floating association, others are plants of small stature, while the two green species, *viz.*, *Chætomorpha linum* and *Enteromorpha intestinalis* are filamentous forms; and in all of them the thallus lacks differentiation into stipes and fronds.

Tables I to VI give the amounts of the major ions, *viz.*, sodium, potassium, calcium, magnesium, and chlorine, present in the different seaweeds in water-soluble form during the different months of collection. The figures given in the tables represent the quantities of the various elements in the ionic form in the cell fluids of the seaweeds. Unfortunately the data on the seasonal variations in the ionic composition of the water-soluble portion of all the seaweeds considered here could not be gathered and to this extent the data presented are not complete. However they are sufficient to show the distinctive characteristics of each species studied in regard to the manner of absorption of elements.

In Tables I to VI, figures are also given for sodium, calcium, magnesium and chlorine on the dry basis as calculated from values obtained from the wet materials. In order to understand the mechanism of absorption of elements by the algæ the values thus obtained by calculation are compared with those from the analyses of the dry algæ. As may be seen from tables moisture content of the species taken up for study varies between 85 to 92%. Most of this water is not in the combined form but in a free state which may be driven out of the cells by prolonged exposure to the atmosphere. It can be assumed that as the moisture content is so high the volume of the cell fluids will be somewhere about 80 to 90 c.c. per 100 gram of the material. This large amount of water in the algæ may be absolutely necessary for the transport of nutrients from the outer medium to the cells.

Selective Absorption of Individual Elements

It is widely accepted and proved by different workers that the elements present in sea-water have a constant proportion to each other and that this factor undergoes only very little change with zones and seasons. If the

absorption of these elements from the water by the seaweeds were a simple process of sea-water getting into the cell walls the amounts of the elements present in the cell fluids would have borne the same proportions as found in natural sea-water. From an examination of the tables it may be seen that the proportion of the elements in the water and in the water-soluble portion of the seaweeds differ widely. This indicates that selective absorption of ions is taking place in the algæ. The ratios between Na and Cl in sea-water collected from the Palk Bay area is about 6: 11 (unpublished data). This is in good agreement with the generally accepted ratio between the two elements as given by Harvey (1928). But in the water-soluble portion of the algæ examined in the present series the value for the above ratio works out differently. In the case of *A. spicifera* collected in June 1952 (Table I), the ratio between Na and Cl is 266: 2553, while the corresponding values for *L. papillosa* and *S. furcellatum* collected during the same months are 516: 2644 and 845: 2788 respectively. This indicates less absorption of sodium as compared to that of chlorine in the species mentioned above, all of them belonging to the group Rhodophyceæ. It can also be seen that even with individual species there is a variation in the ratio between the two ions absorbed. An exception is, however, found in the case of *P. australis*. The ratios between the two ions in the water-soluble portion of this brown alga are found to be 1214: 2232, 1371: 4035 and 2112: 3946 during the months of June, August and October respectively. These values closely resemble the ratio given for normal sea-water, and hence indicate that the alga shows preference to Na ion unlike the Rhodophyceæ mentioned above. Similarly in the Chlorophyceæ, *E. intestinalis*, the ratio does not vary much from that in sea-water. The low absorption noticed in the case of Rhodophyceæ is more evident in the agar-bearing species, particularly in *G. lichenoides*.

Absorption of Potassium, Calcium and Magnesium

From the data presented in the tables it is evident that potassium and calcium are found in high concentrations in the algæ. In certain cases as for example in *P. australis* collected in June 1952, the amount of potassium present in the water-soluble portion of the alga is more than half of the amount of chloride, whereas in sea-water the ratio between the two ions (Cl: K) is roughly about 1: 55. Comparing the amounts of the three ions K, Ca and Mg absorbed by the algæ it can be noticed that potassium and calcium are absorbed to a greater extent than magnesium, the intensity varying with the type of algæ. This is again added proof that the elements present in the algæ are not in similar proportions as they exist in sea-water,

for in sea-water the quantity of magnesium is more than the other two elements. Thus in the seaweeds there is greater absorption of potassium and calcium while the absorption of Cl is not quite proportional to these. The ionic equilibrium between the cell fluid and the external medium is, probably, kept up by greater absorption of SO_4 by the algæ.

Comparison of the over-all ionic composition of the seaweeds (as shown in the graphs) and the calculated values given in Tables I to VI show the following: The chloride values as calculated are, in all cases, higher than the values obtained from the actual analyses of the dry algal samples. This is explained by the fact that a portion of the chloride is lost during incineration. The results also indicate that all the chloride absorbed by the algæ are in an ionic form and not in organic combination. In the case of sodium also the amount found in the ionic form in the water-soluble portion is equivalent to the total quantity found in the entire algæ. It has to be assumed that these elements do not come under the group of "essential nutrients". Sodium and chlorine are present in the form of soluble salts in the sea-water from which the algæ have to absorb certain "essential nutrients"; and naturally to keep up the osmotic balance a certain amount of the sodium and chloride ions also will be absorbed. In the present series of analyses no evidence has, therefore, been obtained as to prove the value of Na as a nutrient in the algæ. It can, however, be observed that Na acts as conserver of potassium, as an inverse relationship has been found to exist between the Na and K contents in certain species of the seaweeds.

It has already been indicated that accumulation of K takes place in the algæ. But it may also be seen that absorption of K is greater in the young and growing plants collected during December and subsequent months. This is in agreement with the findings of early workers in regard to land plants. Evidently potassium also does not undergo any stable organic combination in the seaweeds as almost the entire amount of potassium in the seaweeds exists in the water-soluble form. Janssen and Bartholomew (1929) working on the potassium supply in higher plants have observed that potassium acts as catalyst in the synthesis of carbohydrate, without itself undergoing combination. It has to be assumed that a similar role is played by the element in seaweeds also.

In the case of calcium and magnesium it is observed that only a portion of the total amount is present in the water-soluble inorganic form in all the species studied. The proportion of the water-soluble inorganic form is maximum in the young plants collected in December, the amount becoming less and less as the weeds grow and attain maturity. This suggests that a

certain amount of Ca and Mg absorbed by the plants is used up in the organic complex of the seaweeds. Evidence has been given by other workers (Chapman, 1950) showing that agar and algin exist in the form of Ca and Mg compounds. Studies conducted on higher land plants also bring to light the importance and function of these elements. Presence of calcium regulates the entry of antagonistic ions in the cells. Mg, on the other hand, plays an important direct part in the synthesis of chlorophyll. In the seaweeds Ca and Mg may have these functions also besides their function in the synthesis of carbohydrates.

Variations in the Inorganic Elements in the Different Seaweeds with Seasons and Different Growth Stages

Moisture content.—The moisture content of the seaweeds follows the same pattern in all the species with slight exceptions in certain cases. The maximum moisture content is found in samples collected during November-December. At this period the samples obtained were all young and non-fruiting. The rise in the moisture content starts from August-September, reaches a maximum in December (Figs. 1 to 11), is followed by slow decrease in the values giving the minimum in April-July. This change appears to follow the growth changes in the seaweeds rather than the changes in the seasons. New growth of the algæ starts with the few light showers in August, more rapid growth taking place after the moderate rains in October. In *C. linum* the maximum moisture content was 91·2% in May, with the minimum value in December. The behaviour of the above green species is found to be different from that of the other species. This may be due to the fact that the alga is obtained as drift weed and the sample may not be representative of the growth during the month in which it is collected. The maximum and minimum moisture content of the other algæ are as follows: *G. lichenoides* 89·3% in December and 84·9% in August; *E. intestinalis*, 92·8% in December and 86·6% in September; *A. spicifera* 92·0% in June and 86·0% in January; *H. musciformis* 86·0% in February and 91·0% in June; *L. papillosa* 93·0% in December and 84·0% in August; *S. filiforme* 88·0% in December and 85·0% in June; *S. furcellatum* 89·0% in April and 83·0% in November.

Ash.—The ash content of the algæ taken up for study do not seem to undergo wide variations except in the case of *R. intricata* which belongs to the group of Phæophyceæ. As the place of collection is near the equatorial line there is no marked change between summer and winter so much so that the activity of the plants will be more or less the same throughout the year. Hence there is no likelihood of marked changes in the mineral contents of

the seaweeds unlike that observed in the case of seaweeds of the temperate regions (Black, 1948). This author records a maximum ash content of 43% in the fronds of the sub-littoral seaweeds of Scotland in the month of May and a minimum of 13% in November. But in the present experiments the values are higher especially in regard to the minimum ash content; and the differences between the minimum and the maximum values are very low.

The total ash content in *C. linum* is maximum in May (39·7%) and minimum in December (28·0%); *G. lichinoides* 43·4% in May and 36·2% in February; *C. dasyphylla* 48·4% in September and 32·0% in October; *E. intestinalis* 57·4% in June and 21·6% in February; *A. spicifera* 41·3% in April and 27·8% in June; *L. papillosa* 61·6% in May and 40·0% in December; *S. filiforme* 34·8% in July and 20·6% in February; *S. furcellatum* 39·0% in October and 24·0% in June; *R. intricata* 29·4% in April and 16·0% in May, and *P. australis* 50·0% in May and 30·0% in October.

Except for the floating association species, and *S. furcellatum* the seaweeds show maximum ash content in samples collected in May and the minimum in those collected in the months of October to January. This period corresponds with the two fairly distinct growth stages of the algæ.

It may also be observed that in certain cases there are two maximum and two minimum values for the ash contents, and of these only the more conspicuous are given above. Thus for example *G. lichenoides* exhibits a maximum ash content of 43·4% in May followed by a minimum value of 38·6% in October. Another maximum (42·8%) appears in December followed again by a minimum (36·2%) in February. Such a phenomenon is also observed in *C. dasyphylla*, *A. spicifera*, *L. papillosa*, *H. musciformis* and *S. furcellatum*. This may normally be taken to mean that growth of the algæ is swift, that they attain maturity within a period less than 12 months and that fresh growth takes place more than once in an year in most cases; but as no distinct regularity is seen in the data gathered the above facts cannot be definitely proved. At the same time if only the more distinct and regular changes are taken into consideration they will agree with the findings of other workers, like Black (1948) who observes that the fronds of *Lainaria* sp. are annual features.

Distinct minima in the Na, K, Ca, Mg and Cl contents are found between the months of August and September, the amounts increasing afterwards from December to May or June and giving the maxima in one of these months. This indicates increased absorption of the elements during the early stages of growth of the plants after the rainy season with considerably

limited growth during the latter part of the summer by which time the algæ attain maturity and disappear.

It is also seen from Figs. 1 to 11 that the changes in the absorption of potassium is more marked than any other element. In certain species especially *R. intricata* which belongs to the group of brown seaweeds the potassium content increases to 4·4% of the dry matter.

Trace Element Content of the Algæ

In the present series of experiments only manganese, iron, molybdenum, copper, iodine, zinc and boron have been studied. The results obtained for these elements in eight monthly collections are given in Tables VII to XIV. The quantities of the algæ collected during the intervening months were not sufficient for a complete analysis. However the data obtained give a conclusive idea of the seasonal changes in these constituents.

(i) *Iron.*—There is considerable difference between the three groups of alga, Chlorophyceæ, Phæophyceæ and Rhodophyceæ in regard to their iron contents. The iron content of the Chlorophyceæ is comparatively lower than that of the other two groups, the Phæophyceæ containing the maximum (Table XII). Besides this there are also differences in the iron contents of individual species in the same group. *C. linum* contains a maximum of 217 mg. Fe./kg. in September while the quantity falls down to 42 mg. Fe/kg. in April. In *E. intestinalis*, the other Chlorophyceæ investigated, the iron content varies from 140 mg. in October to 38·6 mg. in April. In regard to the Rhodophyceæ, the variation in iron content in the agar-bearing species, *G. lichenoides*, *C. dasyphylla*, *H. musciformis*, *S. filiforme* and *S. furcellatum*, is most marked, in all instances the values reaching the maxima during September to October. This contrasts with the variations in the major ions in our series of seaweeds, which showed minimum values during the months of August, September and October.

The highest value for iron content obtained in the present studies is 504 mg./kg. in the case of *Padina australis* in October, while much higher values (2040 mg. for *Pelvetia canaliculàta*, 3380 mg. for *Fucus spiralis*, 1150 mg. for *Ascophyllum nodusum* and 1260 mg. for *Laminaria digitata*) have been recorded by Black and Mitchell (1952). However it is evident that the algæ require iron for its growth and metabolism.

(ii) *Copper.*—The amount of copper present in the algæ is comparatively lower than that reported by workers like Oy (1940) and Black and Mitchell (1952). In some of the collections copper was absent or present only in traces; and in such of those collections where the presence of copper

was detected and estimated no definite regularity was observed so that a seasonal correlation could not be obtained. The maximum copper content (12·0 mg./kg.) was found in *A. spicifera* during August and October (Tables X and XII). In the case of the green species, *C. linum* and *E. intestinalis*, the variation of the copper content is only between 1·8 mg. and 5·0 mg./kg. dry weight. In most of the collections of *L. papillosa* copper is present only in traces while in *G. lichenoides* a maximum of 10 mg. is found in August.

(iii) *Manganese*.—Manganese shows wide variations in the species studied. In all the cases the maximum values are obtained in the collections taken in August and the minimum in April. The increase is more marked in the red species of seaweeds, especially in those containing agar. *G. lichenoides* shows a manganese content of 550 mg./kg. during August. This value is quite comparable to the figures given by other workers. Oy (1940) records a manganese content of 100 to 130 mg./kg. in the Fucaceæ, *F. vesiculosus* and *F. serratus* and Black and Mitchell (1952) recorded a maximum of 800 mg./kg. in *F. serratus* collected in January, while in the Laminariaceæ examined by them the usual range recorded is between 30 and 160 mg./kg. Comparing these values it can be observed that the manganese content in the Indian seaweeds is greater than that of the temperate species examined by the above authors. It is found that almost 60% of the total manganese exists in the water-soluble form in the young plants (Table XV) while in the mature and fruiting plants the quantity of water-soluble manganese comes down to 30%. There is practically very little difference between the cold and hot water-soluble portions in any one of the algæ.

(iv) *Boron*.—The variation in the boron content of the seaweeds follow the same course as manganese, giving maximum values in August. In the brown species the maximum value obtained is 9·0 mg./kg. of dry seaweed while in the greens the values are still less. As in the case of manganese the agar-bearing Rhodophyceæ contain the maximum amount of boron (12·8 mg. B in *G. lichenoides*, 7·5 in *H. musciformis* and 8·6 in *S. furcellatum*). Igelsrud, Thompson and Zwicker (1938) record values between 4·2 and 14·9 mg. B/kg. in marine algæ examined by them, while Oy (1940) found up to 100 mg./kg. in Fucaceæ. Unfortunately Black and Mitchell do not seem to have analysed the seaweeds for their boron contents. In studying the partition of the element in the seaweeds it has been observed that only a very small quantity, generally between 10 and 14%, exists in the water-soluble portions of the algæ examined (Table XVI).

(*v*) *Molybdenum.*—The usual range of molybdenum has been found to be between 0·05 and 0·1 mg./kg., but a maximum value of 0·24 has been found in *G. lichenoides* collected in August. Here again the quantity varies from month to month and from species to species giving maximum values in the month of August. Much higher values for the molybdenum content of brown seaweeds have been given by Black and Mitchell (1952) and these values usually vary between 0·03 and 1·3 mg./kg.

(vi) *Iodine.*—The two species of Chlorophyceæ examined contained the lowest amounts of iodine, some of the collections containing no iodine at all. The red species show very wide variations among themselves in regard to iodine content, species like *A. spicifera* and *L. papillosa* giving the least values. The agar-bearing seaweeds show comparatively higher iodine contents but these are quite moderate and much lower than the iodine contents of the two brown species. In all these cases the maximum values were recorded in February while the minimum were recorded in August, except in the case of *S. furcellatum* which gave maximum value (357 mg. I/kg.) in August. The values obtained for these Indian seaweeds are, however, lower than that reported by Black (1948 *a*, *b*; 1949) who recorded between 0·1 and 1·0% iodine in the dry matter of the brown seaweeds.

(vii) *Zinc.*—Not much seasonal variation is found in the zinc content of the seaweeds though a slight increase is observed in the collections made in February. But the variations between the zinc content of the different species are quite apparent—the green forms giving the least values while the red species *G. lichenoides* and *H. musciformis* contain the maximum zinc content. On an average the zinc content varies between 10 and 80 mg./kg. and this is in agreement with the values reported by Black and Mitchell.

Summary

A study of the seasonal variations in the mineral contents of eleven species of seaweeds common to the Palk Bay area has been made. The species studied belong to the three major groups, Chlorophyceæ, Rhodophyceæ and Phæophyceæ.

The data on the water-soluble constituents indicate that selective absorption of particular elements takes place in the algæ resulting in the accumulation of that element. There is evidence that some of the elements like Na and Cl absorbed by the algæ remain almost completely in the inorganic form. Among the major elements the absorption of potassium is found to be the maximum especially in the brown seaweeds.

It is observed that almost all the elements estimated vary not only with species but with seasons and different growth stages of the plants. An

effort has been made to determine the growth stage of the plants by microscopic examination and to correlate with the variations in the chemical constituents. The variations in the dry matter, ash, Na, K, Ca, Mg, Cl, etc., in all the eleven species, have been studied and their significance in the growth cycle of the plants discussed. Similarly the amounts and variations of some of the trace elements in the algæ have also been studied. Phæophyceæ contain the maximum amount of iron, while Chlorophyceæ contain the least. The amount of iron varies considerably with species but in all cases the maximum quantity is found in the mature plants. Copper is present only in small quantities. Manganese, boron and molybdenum are found more in the agarophytes like *G. lichenoides* and reach a maximum in mature plants collected in July to August. As against this the iodine content is found to be maximum in collections made in February when the plants are young and non-fruiting.

An attempt has also been made to follow the partition of the two important trace elements, Mn and B, in the different seaweeds. It is found that in the young plants a major portion of the Mn exists in a water-soluble form while the percentage decreases as the plant grows. In regard to boron only very small quantities are found in the water-soluble form irrespective of whether the plant is mature or young.

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THE MICRO-STRUCTURE OF COALS FROM THE JANGALGALI AND KALAKOT AREAS IN THE JAMMU PROVINCE

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Introduction

The economic importance of the Jammu coalfields first attracted attention about the year 1900. The nature and occurrence of these coals which are regarded as Lower Eocene in age has been studied from time to time by the officers of Mining Department of the Jammu and Kashmir State and also by other geologists. These coals have been generally described as semi-anthracitic with a low volatile content but a high percentage of ash, and some coals are known to exhibit coking properties. The coals generally appear to contain a high percentage of sulphur. Most of the coals are friable and show a foliated structure. The total output of coal recorded from Kashmir between 1940 and 1950 was 35,487 tons.

It is important that a systematic survey of the coal-bearing formations is made with a view to ascertain the economic value of these coals and their possible utilization.

The present work includes a description of the nature of the microscopic constituents observed in coals of the widely separated areas of Jangalgali and Kalakot. A representative set of specimens from these localities was made available through the help of Mr. T. R. Bhatia, Lecturer in Geology, G. M. College, Jammu, who has visited these areas for geological fieldwork. I am thankful to Mr. Bhatia for sending me his collection for study.

Geology of the Area

The geological account of the area including the Sangarmarg and Mehowgala coalfields was given by T. D. La Touche in 1888. Simpson (1904) published an account of the Jammu coalfields in the *Memoirs of the Geological Survey of India.* Middlemiss (1929) studied some of the coalfields in detail and his account of the Kalakot, Metka, Mahogala and other coalfields is published in the *Mineral Survey Reports of Jammu and Kashmir Government.*

The geological account which follows is based mainly on the works of Simpson and Middlemiss.

The coal measures of Jammu Province do not appear to have been formed in the same way as the Lower Gondwana coals, in gently inclined basins or troughs thrown down by faulting. These coals, like some other Tertiary coal deposits in the Salt Range, Baluchistan, Sind, etc., are closely associated with foraminifera-bearing shales and limestones suggesting that the coals were in many cases deposited very close to the seacoast probably in estuaries and marshes.

These coal measures occur on the north-east and south-west flanks of elongated domes or arches and are usually inclined at moderate to steep angles outwards from the dome areas. On the flanks of the domes the coal-bearing strata of Eocene age are overlain by the Murree series, while in the inner parts the underlying Great limestones crop out.

The upper Coal Measures which are about 120 feet thick are overlain by about 400 feet thick Nummulitic series of Eocene age and the Murree series of upper Tertiary age. They are underlain by the Bauxite series, 4 to over 24 feet thick, below which is first the Breccia 20 to 30 feet thick and then the Great limestone of unknown thickness. The Breccia has probably been formed by disintegration of the Great limestone. Its age is not definitely known. There is no definite evidence of age of the Great limestone, and it is probably older than Trias. The limestone over 1,500 feet thick was doubtfully referred to the Kioto limestone of Spiti. Sometimes the Great limestone is identified with the Sirban limestone of Kaghan valley and also

with the Krol limestone of the Simla-Chakrata area (Wadia, 1953). The Bauxite series which is probably of Lower Eocene age is known to pass laterally into hard coal. The coals associated with this series are known as the Lower Coal Measures as distinct from the coals occurring in the Upper Coal Measures.

The geological sequence (Middlemiss, 1929; Gee, 1945) is shown in the table below:—

Nimadric beds (Murrees and Siwaliks)	..	Miocene and younger.
(unconformity)		
Nummulitic limestones and shales	..	About 400 feet (Eocene).
Probably Lower Eocene — Upper Coal Measures	..	Carbonaceous shales with coal seams and ironstone bands. About 120 ft. thick.
Probably Lower Eocene — Bauxite series with Lower Coal Measures	.. ..	Bauxite, bauxitic clays and Kaolin with local ironstone and coal in thick beds at certain places. Thickness 4 to over 24 feet.
Breccia	.. .. ..	Chert fragments mainly in calcareous or siliceous cement; 20 to 30 feet thick. Age unknown.
Great (Riasi) Limestone	..	Massive grey limestone (probably Permian).

The principal areas where coal is known to occur lie in the Riasi (33° 05′: 74° 50′) District of Jammu Province. They are:—

1. The southern or main area, consisting of the large Riasi dome bisected by the Chenab river from north to south, and the three smaller domes of Mahogala, Metka and Kalakot.
2. The northern group of Dhansal-Sawalkot area.
3. The Dandli area in the extreme west.

The Ladda coalfield includes the Jangalgali and Anji valley areas. The south-eastern end of the Ladda coalfield is situated about 21 miles north-north-east of Jammu. The Sangarmarg field is geologically continuous with the Ladda field but the two are separated by the Chenab river. The Dhansal-Sawalkot field extends over a distance of about 12 miles to the east-south-east from Dhansal to Lodhra.

Specimens of coal from the Jangalgali area in the Ladda field and Kalakot area were examined in course of the present study.

The Ladda area

One coal seam of any significance is known to occur in this area. This seam varies in thickness up to 20 feet. In places it dies out entirely. According to Simpson (1913) the only workable portion lies between Ladda and the Anji valley. Here the average thickness of the seam is about 31 inches. About the year 1945 the Mining Department of Jammu and Kashmir State discovered a steeply dipping seam of good coal, 10 feet thick, at Jangalgali, about 42 miles north-north-east of Jammu. Mining by underground methods commenced at Jangalgali and Kalakot about the year 1945.

The Kalakot area

This coalfield is the most westerly of the southern group and its position is in Latitude 33° 13′ and Longitude 74° 25′. The Lower Coal Measures include a seam 17 feet thick. There is another seam about 7 feet thick, but it may be a continuation of the 17-foot seam. Both these seams are hard anthracitic coals of good quality. In the upper part of the Upper Coal Measures, there is a coal seam from 4 to 7 feet thick. The coal of this seam is flaky and powdery with a high ash percentage. Another seam about 2 to 2½ feet thick is known to occur about 40 feet lower in the Upper Coal Measures. This seam is known to yield anthracitic coal of a good quality.

Specimens from Pits No. 1, 2 and 3 at Lain, Kund and Jangalgali top in the Jangalgali area were examined. In hand specimens these coals are very fragile and easily crumble to powder. The coal appears anthracitic in nature and often shows a bright lustre. Its fragile nature suggests that the coal seams were subjected to considerable compression and folding. No banding is seen in any specimen included in the collection.

Specimens of coal from Beragoa in the Kalakot coalfield are compact. It shows a bright silky lustre and a very fine lamination. The lamination tends to disappear in many samples which appear anthracitic. The specimens resemble some Assam coals of Tertiary age.

Microscopic Constituents

The microscopic examination was made on polished surfaces and in thin sections. A great difficulty is experienced in the preparation of thin and polished sections of the Jangalgali coals. These coals are fragile with the result that in the course of its preparation the section develops cracks in large numbers. In case of the Jangalgali coals particularly, it is not easy to make the section sufficiently thin so that only spore materials can be examined with advantage in transmitted light. These coals appear higher in rank as compared to those from Kalakot.

The nature of the microscopic constituents is described below:—

The Jangalgali coals

Examination of polished blocks reveals that they are composed almost entirely of woody tissues which do not show any well-preserved plant structures. A careful examination of thin sections also did not reveal any cellular structure.

Fungal bodies and fungal spores are, however, abundantly preserved and they have produced a widespread decomposition of the plant tissues. The fungi of the groups Ascomycetes and Basidiomycetes are regarded as important agents in the destruction of wood. Their hyphæ absorb the cell contents of xylem and medullary rays. They penetrate the walls and destroy the middle lamella. In this way the whole material is ultimately disintegrated.

The fungal spores observed in these coals are shown in Plate I, Figs. 1–7.

Plate I, Figs. 6 and 7 show fungal bodies known as sclerotia. The nature of sclerotia found in some Indian coals of Permo-carboniferous age has been described by the author (1955 A) elsewhere. The sclerotia figured here resemble very closely *Sclerotites brandonianus* observed by the author in the Tertiary coals of Assam (Ganju, 1955 B). Stach (1934) has observed this species in the brown coals of Germany. The effect of compression is evident in Fig. 7 where the fungal body shows a number of cracks in its thick outer wall.

The spores shown in Plate I, Figs. 1, 3, 4 and 5 resemble teleutospores. The teleutospores are members of Ustilaginales and Uredinales of the group Basidiomycetes. They are parasites on the stems, sporophylls and leaves of vascular plants. The teleutospores may be unicellular or made up of two or more cells, each cell developing independently. They are thick-walled, usually dark coloured, resting spores and develop in considerable quantities either singly or in pairs or in clusters. They are commonly grouped in teleutosori. The spores may bear a stalk or they may be sessile. The generic identification is made on the characters of their shape and septation. The author has observed similar spores to occur in the Assam coals (*loc. cit.*).

Plate I, Fig. 1 shows a slipper-shaped spore having three chambers. The middle chamber is larger in size and is slightly curved and drawn out at one end. Plate I, Fig. 5 shows a spore with four chambers but the septa are much folded and crumpled. The spores in Plate I, Figs. 3 and 4 are three-chambered, and the walls show cracks here and there.

A type of spore which has been found to occur usually in large clusters is shown in Plate I, Fig. 2. These spores are rounded to oval in shape with an empty chamber surrounded by a moderately thick wall which shows in some cases a distinct opening at one end. In some spores the wall is produced into a small neck-like structure near the opening. This is clearly seen in two spores, one in the right-hand upper side and the other in the centre near the upper edge in this figure. These spores are no doubt fungal in origin and are probably teleutospores.

Sideritic matter is quite abundant in these coals and its presence causes much difficulty in obtaining a smooth polish altogether free from scratches.

The Kalakot coals

In thin sections these coals appear distinctly clarainy in nature showing thin strips of vitrain in a ground mass which is largely composed of finely disseminated fusain material. Balls of fusain are conspicuous and constitute most of the macro-fragmentary material. Mineral matter comprising largely of siderite and ankerite is fairly abundant.

Plate I, Fig. 8 and Plate II, Fig. 1 show thin sections of these coals. An oval patch of fusain is observed in the upper left-hand side in Plate I, Fig. 8. A broad strip of vitrain is seen running across near the right-hand edge in this figure. It does not reveal any cellular structure. Plate II, Fig. 1 shows a typical coal exhibiting long narrow strips of vitrain placed almost parallel to one another, and finely granular fusain material and small mineral grains scattered in the ground mass. Dark oval or slightly compressed bodies seen occasionally are probably fungal in origin.

Plate II, Figs. 2–7 show polished surfaces of these coals in reflected light under oil immersion. Fig. 2 shows a band of wood in fusain with well-preserved rows of tracheids. An oval ball of fusain revealing clearly its woody nature appears in Fig. 4. A much decomposed woody tissue with fragments of cells scattered here and there is seen in Fig. 3.

Fungal spores are less abundant. The fungal bodies observed in polished sections appear in Plate II, Figs. 5–7 which show sclerotia in various forms. They have a carved outline and are very hard with the result that the surrounding tissues are closely compressed and folded, a fact clearly observed in Fig. 5. In their form and structure some of the sclerotia resemble those observed in the Jangalgali coals.

Summary and Conclusions

Representative specimens of coal from the Jangalgali area in the Ladda coalfield and from the Kalakot coalfield in Jammu Province have been examined for their microscopic constituents.

In its physical appearance the Jangalgali coal is anthracitic and often shows a bright lustre. It is fragile and easily crumbles to powder. The Kalakot coal is compact; it shows a bright silky lustre and a very fine lamination which tends to disappear in some anthracitic varieties.

The microscopic examination was made on thin sections and polished surfaces.

The Jangalgali coals are composed almost entirely of woody tissues which do not show any well-preserved plant structures. Fungal organisms have produced a widespread decay in these tissues. Sclerotia resembling *Sclerotites brandonianus* occur in large numbers. The teleutospores showing three or four chambers are found to occur in various forms. Small rounded or oval spores with a moderately thick wall and showing a distinct neck-like opening at one end are found to occur in large clusters. These are probably teleutospores. Sideritic matter is quite abundant.

The Kalakot coals appear distinctly clarainy showing thin strips of vitrain, often placed in parallel rows, in a ground mass which is largely composed of finely disseminated fusain material. Balls of fusain, usually revealing their structure clearly preserved, are conspicuous and constitute most of the macro-fragmentary material. Mineral matter comprising largely of siderite and ankerite is widely distributed in the ground mass. The fungal spores are less abundant, but sclerotia have been observed to occur commonly in these coals also.

The microscopic constituents reveal that the coals have generally undergone much compression and folding, the Jangalgali coals more so than those of Kalakot.

In the nature of their fungal organisms these coals appear to resemble some Tertiary coals of Assam.

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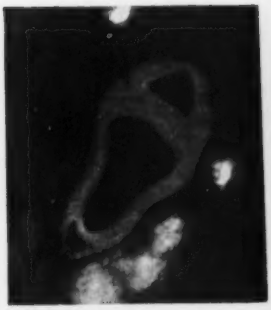
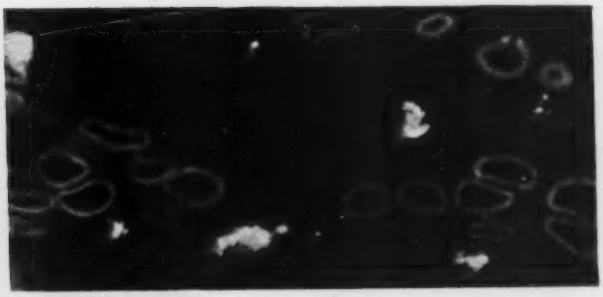


Fig. 1. (×1200).



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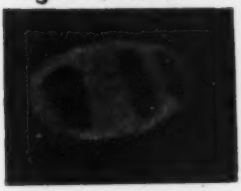

Fig. 3. (×1200).

Fig. 5. (×1200).

Fig. 6. (×1200).

Fig. 4. (×740).

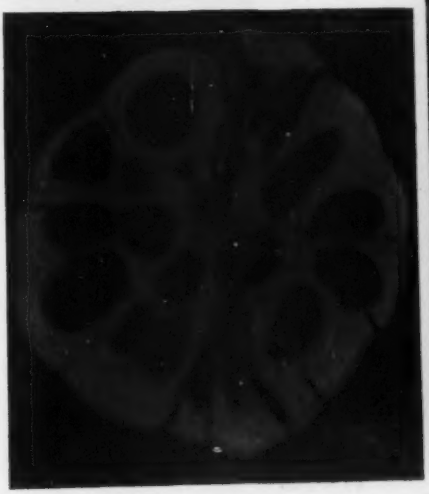

Fig. 7. (×1200).

Fig. 8. (×165).

Fig. 1. (×165).

Fig. 2. (×280).

Fig. 3. (×280).

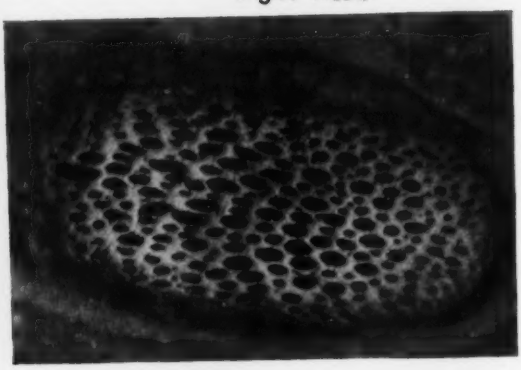

Fig. 4. (×280).

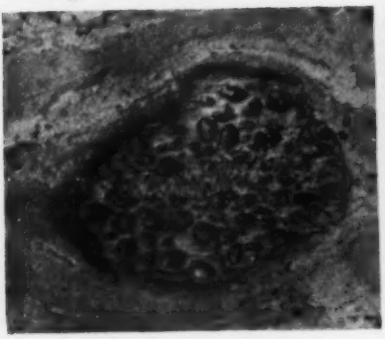

Fig. 5. (×360).

Fig. 6. (×380).

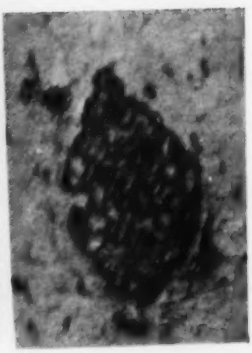

Fig. 7. (×380).

Explanation of Plates

Plate I

(All Figures are from thin sections)

Fig. 1. A slipper shaped teleutospore showing three chambers. The middle chamber is larger in size and is slightly curved and drawn out at one end.

Pit. No. 3, Jangalgali coal (× 1200).

Fig. 2. A segregation of round and oval spores which are probably teleutospores. They show an empty chamber enclosed by a moderately thick wall. Two spores, one in the right-hand upper side and the other in the centre near the upper edge, show a small neck-like opening at one end.

Pit. No. 3, Jangalgali coal (× 740).

Fig. 3. Teleutospore showing three chambers. The thick septa shows a crack.

Pit. No. 3, Jangalgali coal (× 1,200).

Fig. 4. Teleutospore showing three chambers. The walls show cracks formed due to compression.

Pit. No. 3, Jangalgali coal (× 740).

Fig. 5. Teleutospore with four chambers showing much folded and crumpled septa.

Pit. No. 3, Jangalgali coal (× 1,200).

Fig. 6. Sclerotia resembling *Sclerotites brandonianus*.

Pit. No. 3, Jangalgali coal (× 1,200).

Fig. 7. Sclerotia resembling *Sclerotites brandonianus*. The thick outer wall shows a number of well-developed cracks formed due to compression.

Pit. No. 1, Jangalgali coal (× 1,200).

Fig. 8. Clarain-like coal showing an oval patch of fusain in the upper left-hand side. A broad strip of vitrain is observed running across near the right-hand edge.

Kalakot coal (× 165).

Plate II

(Fig. 1 is from a thin section; all other figures are from polished sections in reflected light under oil immersion.)

Fig. 1. Clarain-like coal exhibiting narrow strips of vitrain placed in almost parallel rows. Finely granular fusain material and mineral grains are scattered in the ground mass. Small dark oval bodies are probably fungal in origin.

Kalakot coal (× 165).

Fig. 2. A band of wood in fusain with well-preserved rows of tracheids.

Kalakot coal (× 280).

Fig. 3. Much decomposed woody tissue with fragments of cells scattered here and there.

Kalakot coal (× 280).

Fig. 4. An oval ball of fusain revealing clearly its woody structure.

Kalakot coal (× 280).

Fig. 5. Sclerotia showing a carved outline. The surrounding tissue is clearly folded and compressed.

Kalakot coal (× 360).

Fig. 6. Sclerotia in a much decomposed woody tissue.

Kalakot coal (× 380).

Fig. 7. Same as in Fig. 6.

Kalakot coal (× 380).

INHERITANCE OF LEAF RUST REACTION IN CROSSES OF VULGARE WHEATS

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Introduction

Many investigators had noted that varieties of wheat susceptible to leaf rust, *Puccinia rubigo-vera tritici* (Erikes. and Henn.) Carl., in the seedling stage may develop resistance in later growth stages. Mains *et al.* (1926) found in the F_2 of the cross of Fulcaster and Kanred when tested to race 9, resistant and susceptible seedlings, in the ratio of 1:3. Homozygous resistant and susceptible plants maintained their resistance and susceptibility respectively in all stages of the growth of the plants, but the heterozygous plants became progressively more resistant in the shooting and heading stages, reversing the ratio of resistant and susceptible individuals from 1:3 to 3:1.

For a comprehensive review of literature on the inheritance of leaf rust reaction the reader is referred to Ausemus (1943), Wu (1950) and Hashim (1951). The present study seeks to determine the mode of inheritance of seedling and adult plant reaction to physiologic race 126 of leaf rust which comprised the bulk of total isolates in the spring wheat region of the United States, in the cross of Frontana, a Brazilian wheat which is resistant to the above race both in the seedling and adult plant stages with the susceptible spring wheat variety, Thatcher. An attempt is also made to analyse genetically the association which exists between seedling and adult plant reaction.

Experimental

A single F_2 culture of the cross of Frontana (C.I. 12470) with Thatcher (C.I. 10003) together with the parents were planted in the greenhouse in the autumn of 1950. Race 126 of leaf rust used in these experiments was obtained from the Division of Plant Pathology and Agricultural Botany of the University of Minnesota. When the seedlings were in the one-leaf stage about three inches high they were sprayed with water and brushed with pots of Thew wheat on which the race was increased. The inoculated seedlings were then incubated for twenty-four hours. For the study of adult

plant reaction the inoculation was done at the six-leaf stage. Notes on the leaf rust reaction were taken about two weeks after incubation. Seedling and adult plant reactions were read according to the types of infection described by Mains and Jackson (1926). Type 0; 1, 2, and X were reported as resistant and type 3 and 4 as susceptible. An effort was made to conduct all experiments at the same temperature, *viz.*, 70° F.

Results and Discussion

Inheritance of Seedling Reaction

A single F_2 culture of Thatcher × Frontana and the parents were inoculated at the seedling stage with race 126 of leaf rust. The Frontana parent showed type 1 infection and Thatcher was susceptible with type 4 pustules. The F_2 seedlings were classified into the parental classes as shown in Table 1.

Table I

Frontana, Thatcher and the F_2 generation of their cross

Classified for seedling reaction to race 126 of leaf rust

Parent or F_2	Number of parent or F_2 plants		Total
	Resistant	Susceptible	
Thatcher P	..	20	20
Frontana P	20	..	20
Thatcher × Frontana F_2 ..	50	238	288

A calculated P value between 0·70 and 0·50 for goodness of fit to a 3:13 ratio was obtained, the calculated numbers being 54 resistant and 234 susceptible. This segregation suggests the hypothesis that Frontana has a pair of factors for resistance to race 126 and Thatcher carries a dominant susceptibility factor which is epistatic to a resistance factor of Frontana.

On this basis the genotypes of the parents for seedling reaction to race 126 of leaf rust would be

Frontana .. .. RR ss
Thatcher .. .. rr SS

The genotype of the F_1 would be Rr Ss and the phenotypic ratio expected in the F_2 would be 3 resistant: 13 susceptible, only the genotypes RR ss and

Rr ss having the epistatic factor in recessive condition would produce the resistant reaction. The F_3 breeding behaviour would be as follows:

F_2 genotype Frequency	F_2 Genotype	F_2 Phenotype	F_3 Breeding Behaviour
1	RR SS	Susceptible	Breeds true for susceptibility.
2	RR Ss	,,	Segregates for resistance and susceptibility in 1:3 ratio.
2	Rr SS	,,	Breeds true for susceptibility.
4	Rr Ss	,,	Segregates for resistance and susceptibility in 3:13 ratio.
1	RR ss	Resistant	Breeds true for resistance.
2	Rr ss	,,	Segregates for resistance and susceptibility in 3:1 ratio.
1	rr SS	Susceptible	Breeds true for susceptibility.
2	rr Ss	,,	Do.
1	rr ss	,,	Do.

Based on this hypothesis of 16 F_3 lines 7 would breed true for susceptibility, 8 would segregate for resistance and susceptibility, and 1 would breed true for resistance.

As the average number of seedlings tested for each F_3 line was 20 it was not possible to distinguish between a segregation ratio of 13 susceptible : 3 resistant and 3 susceptible : 1 resistant, but it was possible to distinguish 1 susceptible: 3 resistant segregations from the others when the same culture of race 126 of leaf rust which was used to inoculate the F_2 plants in the previous experiment was used to inoculate 74 F_3 lines of Thatcher × Frontana (Table II).

The results indicate a satisfactory agreement with the hypothesis although there is an excess of susceptible lines and a deficiency of segregating ones.

Inheritance of Adult Plant Reaction

The parents and a single F_2 culture of Thatcher × Frontana consisting of 288 plants used in the previous experiment (Table I) were inoculated with

TABLE II

X^2 test for goodness of fit for 74 F_3 lines of Thatcher × Frontana

Classified for seedling reaction to race 126 of leaf rust in the greenhouse calculated on the basis of 7 susceptible : 6 segregating 13 susceptible to 3 resistant or 3 susceptible to 1 resistant : 2 segregating 1 susceptible to 3 resistant : 1 resistant. Greenhouse test autumn 1950

	Observed	Calculated	$\frac{(O-C)^2}{C}$
Number of lines breeding true for susceptibility	37	32·37	0·4058
Number of lines segregating for susceptibility and resistance in 13 : 3 and 3 : 1 ratio ..	25	27·75	0·2725
Number of lines segregating for susceptible and resistant reaction in 1 : 3 ratio	8	9·25	0·1689
Number of lines breeding true for resistant reaction	4	4·625	0·0844
Total ..	74	..	0·9316
$X^2 = 0·9316$ D.F. = 3		P = 0·90 − 0·80	

race 126 of leaf rust at the six-leaf stage and classified for their reaction, the data being in Table III.

TABLE III

Reaction of 288 F_2 plants of Thatcher × Frontana cross and parents to race 126 of leaf rust at the six-leaf stage

Tested in the greenhouse in the winter of 1951

Parent or F_2	Number of parent or F_2 plants			Total
	Resistant	Mesothetic	Susceptible	
Thatcher P	..	..	20	20
Frontana P	20	..	..	20
Thatcher × Frontana F_2 ..	58	149	81	288

The Frontana parent was resistant with mostly 0; to type 1 pustules. Thatcher was susceptible with type 4 pustules. The F_2 plants were resistant like Frontana, had mesothetic reaction or were susceptible like Thatcher (Table III). A X^2 test for goodness of fit to 1 : 2 : 1 ratio was made on the assumption that the heterozygotes showed a mesothetic reaction. The calculated P value was between 0·20–0·10 and suggests segregation for a single factor pair. The segregation is in agreement with the hypothesis that Frontana possesses a pair of factors for resistance to race 126 of leaf rust at the adult plant stage, Thatcher having a recessive gene resulting in complete susceptibility. The heterozygotes show incomplete dominance of resistance resulting in the mesothetic reaction.

Linkage between Seedling and Adult Plant Reaction

Table IV shows the reaction of 288 plants at the seedling and adult plant stage (six-leaf) to race 126 of leaf rust in the greenhouse.

TABLE IV

Reaction of 288 *plants at the seedling and adult plant stages to race* 126 *of leaf rust*

	Resistant adult	Susceptible adult	Total
Resistant seedling	*a* 47	*c* 3	50
Susceptible seedling ..	*b* 160	*d* 78	238
Total ..	207	81	288

The X^2 test for independence shows a highly significant association between the seedling and adult plant reaction. The actual X^2 being 14·6513 with a P value of less than 0·01.

Having detected linkage between the seedling and adult plant reaction it was thought necessary to measure the strength of linkage. Product method tables are not available for such a complex type of segregation. Hence new formulæ had to be derived using the method of maximum likelihood.

Derivation of Maximum Likelihood Formula for (3 : 13) (3 : 1) *Segregation*

Segregation for reaction to race 126 of leaf rust at the seedling stage could be explained by a factor S which was epistatic to the resistance factor R resulting in a ratio of 3 resistant to 13 susceptible plants in the F_2 generation. When the same F_2 plants were inoculated at the six-leaf stage a

ratio of 3 resistant to one susceptible was obtained and new combination types appeared, *viz.*, some plants which were susceptible at the seedling stage became resistant at the six-leaf stage and *vice versa*. A second factor R_2 is assumed for resistance at the six-leaf stage with S assumed to be epistatic only to R, not to R_2. In order to calculate linkage values it is necessary here to make a few assumptions. There could be two possibilities: (1) The gene for resistance at the six-leaf stage R_2 could be linked with the S factor which is epistatic to the gene for resistance at the seedling stage or (2) the gene for resistance at the six-leaf stage R_2 could be linked with the gene R for resistance at the seedling stage.

Maximum likelihood formulæ were derived assuming no difference in recombination in the two sexes. For the mathematical procedure in the derivation of the formulæ for the F_2 phenotypes the reader is referred to Fisher and Balmukund (1928) and Mather (1938). The standard error formulæ were calculated by a method given by Mather (1938).

Application of the Formulæ to the Data

Based on the first assumption, *i.e.*, the factor R_2 is linked with the S factor the maximum likelihood formula is:

$$6p\left[-\frac{a}{3-3p^2}+\frac{b}{9+3p^2}+\frac{c}{3p^2}-\frac{d}{4-3p^2}\right]=0$$

The scores when different values of p were substituted in the formula were:

Recombination Fraction p	Scores
·25	−5·632800
·20	−7·345587
·235	−3·592268
·23	−0·658900
·225	−0·626598
·2275	−0·138626
·2265	−0·240704
·227	−0·112234

The score nearest zero was obtained when a p value of ·227 was substituted. To determine the standard error, the p value of ·227 was substituted in the formula

$$i=\frac{36p^2}{16}\left[\frac{1}{3-3p^2}+\frac{1}{9+3p^2}+\frac{1}{3p^2}+\frac{1}{4-3p^2}\right]$$

giving a value of ·83356 for i (information). Using this in the formula S.E.$p=\sqrt{1/n\times i}$ gives a standard error of 6·4 per cent. for the recombina-

tion fraction. Therefore according to the above assumption the factor for adult plant resistance R_2 was linked with the S factor epistatic to the R factor for resistance at the seedling stage with a recombination value of $22{\cdot}7 \pm 6{\cdot}4$ per cent.

Based on the second assumption, *i.e.*, the factor R_2 for adult plant resistance was linked to the R factor for resistance at the seedling stage, the maximum likelihood formula is

$$2p\left[\frac{a}{2+p^2} - \frac{b}{10-p^2} - \frac{c}{1-p^2} + \frac{d}{3+p^2}\right] = 0.$$

The scores when different values of $(I - p)$ (since the data are in coupling) were substituted in the formula were:—

Recombination Fraction $(I - p)$	Scores
·10	−7·39566
·08	−4·21728
·085	−1·83065
·0825	−0·74699

The score nearest to zero was obtained when a $(I - p)$ value of ·0825 was substituted. To determine the standard error the p value of ·9175 was substituted in the formula (p is the parental combination):

$$i = \frac{4p^2}{16}\left[\frac{1}{2+p^2} + \frac{1}{10-p^2} + \frac{1}{1-p^2} + \frac{1}{3+p^2}\right]$$

giving a value of 1·482148 for i (information). Using this in the formula S.E.$p = \sqrt{1/n \times i}$ gives a standard error of 4·8 per cent. Therefore according to the second assumption the resistance factors for seedling and adult plant reaction, R and R_2 are linked with a recombination value of $8{\cdot}25 \pm 4{\cdot}8$ per cent.

These calculations indicate that all the three factors R, R_2 and S are linked, but the linkage between R and S is loose amounting to independent assortment.

CONCLUSIONS

It is generally accepted that a variety of wheat, when resistant in the seedling stage to a particular physiologic race of stem or leaf rust, usually is resistant all throughout the life of the plant. This does not seem to be true in the hybrid generation of Thatcher and Frontana when it was tested to race 126 of leaf rust in the seedling and adult plant (six-leaf) stages

at least under greenhouse conditions. The study has shown that those plants which are resistant at the seedling stage may or may not be resistant at the adult plant stage. Seedling and adult plant reactions are governed by different genes but there is a linkage between the two. There were some plants recovered which were susceptible at the adult plant stage even though they were resistant at the seedling stage. Hence it is suggested that the breeding material should be tested both in the seedling and adult plant stages.

Summary

Based on F_2 data and checked in the F_3 generation for the Thatcher × Frontana cross, the seedling reaction to race 126 of leaf rust was explainable on the basis of an interaction of a dominant factor for resistance from Frontana with a dominant factor for susceptibility from Thatcher, the latter being epistatic to the Frontana factor, the resulting ratio in the F_2 being 13 susceptible : 3 resistant.

Resistance to race 126 of leaf rust at the six-leaf stage appeared to be governed by a single factor pair with incomplete dominance in the Thatcher × Frontana cross.

Maximum likelihood formulæ were derived to measure linkage between six-leaf stage reaction to race 126 of leaf rust, segregating 3 resistant : 1 susceptible and seedling reaction to the same race, segregating 13 susceptible: 3 resistant. The formula for an assumed linkage between the factor for adult plant resistance R_2 and the R factor conditioning resistance at the seedling stage gave a recombination of $8{\cdot}25 \pm 4{\cdot}8$ per cent. The formula for an assumed linkage between the factor for adult plant resistance R_2 and the S factor epistatic to the R factor for resistance at the seedling stage gave a recombination of $22{\cdot}7 \pm 6{\cdot}4$ per cent. Linkage is indicated and all the three factors R, R_2 and S are linked, but the linkage between R and S appears to be loose amounting to independent assortment.

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VARIATIONS IN THE STRUCTURE OF THE NUCLEUS IN LIVING YEAST

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Introduction

There appears to be a large measure of agreement among cytologists regarding the identification of the 'homogeneous body' seen under certain conditions in the living yeast as its nucleus. The divergences of opinion relate only to the nuclear nature of the vacuole (Royan and Subramaniam, 1956). Wager and Peniston (1910) who identified the vacuole of yeast as its nucleus were emphatic (p. 75) that the 'nuclear vacuole' does not take part in sporulation. In the context of the geneticist's limitation of the term 'germ plasm' to the chromosomes (Sharp, 1926, p. 485)—and hence to the nucleus—only that structure which shows organic continuity from one vegetative generation to the next through an intervening sexual phase could be identified as its nucleus. Available evidence indicates (Table I) that such organic continuity could be presumed only in the case of the 'homogeneous body'.

Historical Resumé

In 1844 Nägeli (Wager, 1898) reported having observed a nucleus in living yeast. Wager (1898) remarks: "It may perhaps be doubted if Nägeli had before him the body known to recent observers as the nucleus; but it is interesting to find that under certain conditions the nucleus can be observed in the fresh condition of the cell, and is always in the position indicated by Nägeli" (p. 499).

The rarity of observations on the living nucleus (Table II) would imply that only in some strains of yeast and that under special conditions could the nucleus be observed.

TABLE I

Organic continuity of cell organelles through spores

Name of Investigator	Homogeneous Body		Vacuole	
	Terminology	Role	Terminology	Role
1. Wager and Peniston, 1910	Nucleolus	Takes part in sporulation. (see Figs. 69–71, Wager, 1898)	Nuclear Vacuole	No direct role during sporulation
2. Guilliermond, 1920 ..	Nucleus	Exhibits phenomena suggestive of meiosis	Vacuole	Do.
3. Nagel, 1946 ..	Parvicorp	Do.	Magnicorp	Do.
4. Lindegren, 1952 ..	Centrosome	The centrochromatin could not be differentiated from chromosomes	Nuclear Vacuole	Appears to condense around the centrosome bringing the chromosomes into close contact with it
5. Widra and DeLamater, 1955	Nucleus	Exhibits phenomena suggestive of meiosis	Vacuole	No direct role during sporulation

TABLE II

Record of observations on the nucleus in the living yeast

Name of Investigator	Homogeneous body = Nucleus
1. Nägeli, 1844 ..	.. A little nucleus of whitish mucus, lying on the membrane, regularly in each cell (Wager, 1898).
2. Henneguy, 1896 ..	.. Exhibited a nucleus surrounded by a nuclear membrane and possessing a nucleolus (Wager, 1898).
3. Wager, 1898 ..	.. Under certain conditions the nucleus can be observed in the position indicated by Nägeli.
4. Wager and Peniston, 1910	.. Slightly refringent body in contact with the central vacuole.
5. Henneberg, 1915 ..	.. Could be revealed by 0·25–0·5% acetic acid.
6. Nagel, 1946 ..	.. Rarely visible, but its position could be determined by the slight invagination of the magnicorp.
7. Lindegren and Rafalko, 1950	A more opaque area in the cytoplasm.
8. Townsend and Lindegren, 1953	.. Centrochromatin and centrosome visible in cells suspended in water adjusted to a pH of 3·5 with acetic acid.
9. Mundkur, 1954 ..	.. The nucleus appears as a zone of reduced intensity under the phase contrast microscope.
10. Royan and Subramaniam, 1956	.. Observed in 96–120-hr. wort cultures. It has a membrane and contains formed structures inside.

OBSERVATIONS

It was shown (Royan and Subramaniam, 1956) that the structure identified by Nägeli as the *nucleus* could be observed in living cells from 96–120-hr. cultures of a strain of *Saccharomyces cerevisiæ*. The photographs taken

under ordinary, dark-ground and especially phase contrast illumination (Photos 1–6, Royan and Subramaniam, 1956) would suggest that the nucleus has regions of differing optical density and no uniformity in structure could be presumed from cells observed in the same field. Its description as a 'homogeneous body' by Wager and Peniston (1910) is based on observations under ordinary illumination. The phase contrast microscope is comparatively a recent introduction.

Since with rare exceptions the structure described as the nucleus of yeast by the investigators who preceded Wager (1898) was from stained preparations, it becomes necessary to offer proof that the body identified as the nucleus in living yeast (Royan and Subramaniam, 1956) exhibits similar reactions to fixatives and stains employed by the early students.

A. *Choice of Fixatives.*—Brücke (1861; see Wager, 1898) fixed yeast with iodine and acetic acid but was unable to locate the nucleus. In 1892, Moeller used iodine in potassium iodide as a fixative and stained the cells with hæmatoxylin after mordanting with picric acid. Wager (1898) found Gram's iodine solution to be of immense value in his work. This appears to be the experience of Wager and Peniston (1910), Nägel (1946) and Lindegren (1949). Formaldehyde and acetic acid as suitable components of fixatives were known to the earlier cytologists. Apart from fixing yeast in various concentrations of iodine in pot. iodide, Nagel (1946) compounded a fixative having iodine, pot. iodide, formaldehyde and acetic acid.

The advantage of being able to see the nucleus in the living cell rendered it possible to follow its behaviour during the progress of fixation not only in Gram's solution (I_2 1 gm.; KI 2 gm.; dist. water 300 ml.) but also in a fixative having the following formula: Gram's iodine soln. 90 ml.; neutral formaldehyde 9–12 ml.; glacial acetic acid 1–1·5 ml. Cells from 120-hr. wort cultures were smeared one cell thick on slides coated with a thin layer of Mayer's albumen and fixed in Coplin jars containing the two fixatives. The progress of fixation was followed every ten minutes by mounting the smear under a coverslip in a drop of the fixative.

B. *Effect of Fixation and Hydrolysis.*—Photos 1 and 2 illustrate the appearance of the cells after fixation for 24-hr. in the two fixatives, while photos 3 and 5 are of fixed cells washed in running water for 2–5 min. A longer wash of smears fixed in Gram's soln. makes photography difficult. However, the nucleus could be seen in the cells under phase contrast even after a wash in running water for one hour (Photo 4).

The existence of the structure identified by Moeller (see Wager, 1898), —in yeast cells fixed in Gram's iodine and stained with hæmatoxylin,—as

the nucleus was questioned by some of his contemporaries. Moeller mentions that the staining of the nucleus could be improved by treating the cells with hot water. It is known that hot water removes the metaphosphate present in the cytoplasm (Lindegren, 1949). It was considered desirable to remove the cytoplasmic basophilia by hydrolysis of the fixed material for 8–10 minutes in N HCl at 60° C. (Kurnick, 1955) after a long wash under the tap followed by a rinse in distilled water. Photos 6, 7 and 8 are of hydrolysed cells under ordinary as well as the phase contrast type of illumination.

C. *Effect of Staining.*—The smears were then mordanted in 4% ferric ammonium sulphate for 6–24-hr., washed in running water for 15 minutes and transferred to 0·5% well ripened hæmatoxylin. After being in the stain for 16–48-hr. the smears were carefully destained in 2% iron alum. Photo 9 is that of a glycerine mount during the process of differentiation. As will be seen the body identified as the nucleus by the early workers has a great affinity for hæmatoxylin. Destaining was carefully controlled under the oil immersion lens. The slides were then washed under tap for ½–1-hr., carefully dehydrated through ascending grades of alcohol, cleared in alcohol-xylol mixtures followed by pure xylol and mounted in Canada balsam.

Photo 10 is that of a budding cell where the staining of the body is homogeneous. The vacuole is bereft of any stained structures. There is a crescentic unstained area in the nucleus in Photo 11. The vacuole appears overlaid by cytoplasmic strands stained to the same intensity as the cytoplasm surrounding the vacuole (Photo 11). The nucleus contains a bipartite structure in Photo 12 and the two halves are separated in Photo 13. In Photos 9, 10, 11, 12 and 13 a membrane delimiting the nucleus from the cytoplasm could only be surmised. The nucleus has a distinct stained membrane and encloses a pair of bodies in Photo 14.

It is justifiable therefore to conclude that the body identified as the nucleus in the living cell (Royan and Subramaniam, 1956) is the structure which stains with hæmatoxylin. It appears to be identical with the nucleus described by many of the early investigators (Wager, 1898).

Variations in the Structure of the Living Nucleus

A perusal of the stained preparations (Photos 9–14) would show that there is no uniformity in the structure of the resting nucleus. The same diversity could be observed in living cells when examined under the phase contrast microscope. A clear idea of these variations would be desirable to evaluate the pictures to be expected in stained preparations. The nucleus

is seen as a homogeneous sphere projecting into the vacuole in Photo 15. Its optical density appears similar to that of the cytoplasm and hence it was found difficult to get a good photograph. This condition was, however, rather rare. Even when the nucleus appears as a homogeneous body it could easily be located in the living cell as it is usually denser than the cytoplasm (Photos 16 and 17). Though no formed structures are seen inside, some areas of these nuclei (Photos 16 and 17) appear denser. Attention is invited to the membrane delimiting the nucleus from the cytoplasm (Photos 16 and 17; *cf.* Photo 14). A central grain is clearly visible inside the nucleus in Photos 18–22 and 25–31 reminding one of the nucleolus described by Guilliermond (1920) and some of the early investigators.

While the grains lying just outside the nucleus do not mask the structure in Photos 18, 19 and 21, the two big grains in Photo 22 lying between the nucleus and the vacuole may give the impression that they are actually inside the nucleus. Observations under different foci suggest that these grains are lying outside the nuclear membrane. That grains occasionally lie apposed to the outside of the nuclear membrane could be made out from Photo 23 where the nucleus appears homogeneous. In budding cells the nucleus usually lies near the junction of the bud with the mother cell. It is at the opposite end of the cell in Photo 24.

Photos 25, 26, 27 and 28 are extremely interesting. Apart from the central grain, the nucleus shows denser optical regions at its periphery. Being not uniform these regions give a horse-shoe shape to the nucleus. The nuclear membrane when carefully followed is found to lie outside these patches. The denser regions inside the nucleus have, therefore, to be clearly distinguished from the grains lying outside the nuclear membrane as in Photos 22 and 23.

Considerable attention was devoted to the question whether the structures in Photos 25, 27 and 28 were actually inside the nucleus. It was reasoned as follows. The nucleus in the living yeast is a spherical or oval structure having a distinct membrane (Photos 16 and 17). If the denser areas are outside the nucleus, it would appear from Photo 29 that even in a living cell the nucleus could have a bizarre shape. But if one visualises the nuclear membrane as enclosing the denser areas in Photos 25, 27, 28 and 29, then the nuclei of these cells would be comparable in shape to those illustrated in Photos 16 and 17. Evidence for such a probability is offered by Photo 26 of a stage similar to that seen in Photo 25, but where the nuclear membrane instead of the denser areas is in focus. It has an oval shape. Apart from the presence of grains in photos 18, 19 and 20 there are peripherally dis-

posed denser regions projecting into the nuclear matrix in Photos 30 and 31. Photos 32 and 33 offer conclusive evidence that the pair of darker areas seen inside the nucleus are definitely enclosed by the nuclear membrane. This condition is reminiscent of that illustrated in Photo 14 from stained preparations.

Discussion

Though Nägeli recorded having seen the nucleus in a living cell as far back as 1844, the rarity of such reports later (see Table II) imply that the nucleus may not be visible in all strains of yeasts and that even in the same strain it may be discernible only under particular physiological conditions. In the yeast which forms the subject of this study, the nucleus is clearly perceptible only in 96–120-hr. cultures grown in wort at a temperature of 22–25° C.

The invisibility of the nucleus in the living state in many strains of yeasts imports a certain measure of uncertainty in evaluating the cytological procedures employed to reveal its structure and behaviour. It is in this context that the observations reported in this paper are of interest. There is no uniformity in the architecture of the living nucleus. It ranges from a homogeneous structure to those with clearly formed bodies enclosed by a nuclear membrane. Even in those yeasts where it is said to be visible in the living cell it has been described as a homogeneous body. Mundkur (1954) records that it is optically empty. The phase photographs (15–33) presented in this paper would show that such a conclusion is not capable of a generalization.

The clarity of the nucleus under the phase contrast microscope and the normal variations in its structure in the living state enabled the cells to be kept under observations during the processes of fixation, staining and dehydration (Photos 1–14). The iodine-formol-acetic solution gives a life-like preservation except for the slight reduction in the overall size of the cell. Hydrolysing the smears in normal HCl and staining with iron hæmatoxylin by the long method of Heidenhain reveals only the structures seen in the living nucleus.

When a living nucleus appears homogeneous no further details could be revealed by staining (Photo 10). If on the other hand it contains formed structures, staining reveals the bodies as well as the nuclear membrane. Evidence for the presence of bodies (Photo 14) lying free inside the living nucleus being unmistakable there is no reason why they may not on occasion lie apposed to the inner surface of the nuclear membrane (Photos 11–13). The prerequisite for such a deduction would be the unmistakable occurrence of a nuclear membrane.

SUMMARY

1. The nucleus could be observed in the living cells from a 96–120-hr. culture of a strain of *Saccharomyces cerevisiæ*.

2. It could be followed up during the processes of fixation, hydrolysis, staining and dehydration. In its staining reactions it is identical with the nucleus described by the early investigators.

3. Under the phase contrast microscope there is no uniformity in the architecture of the resting nucleus in living cells. It may be a homogeneous body or may contain denser areas or formed structures inside.

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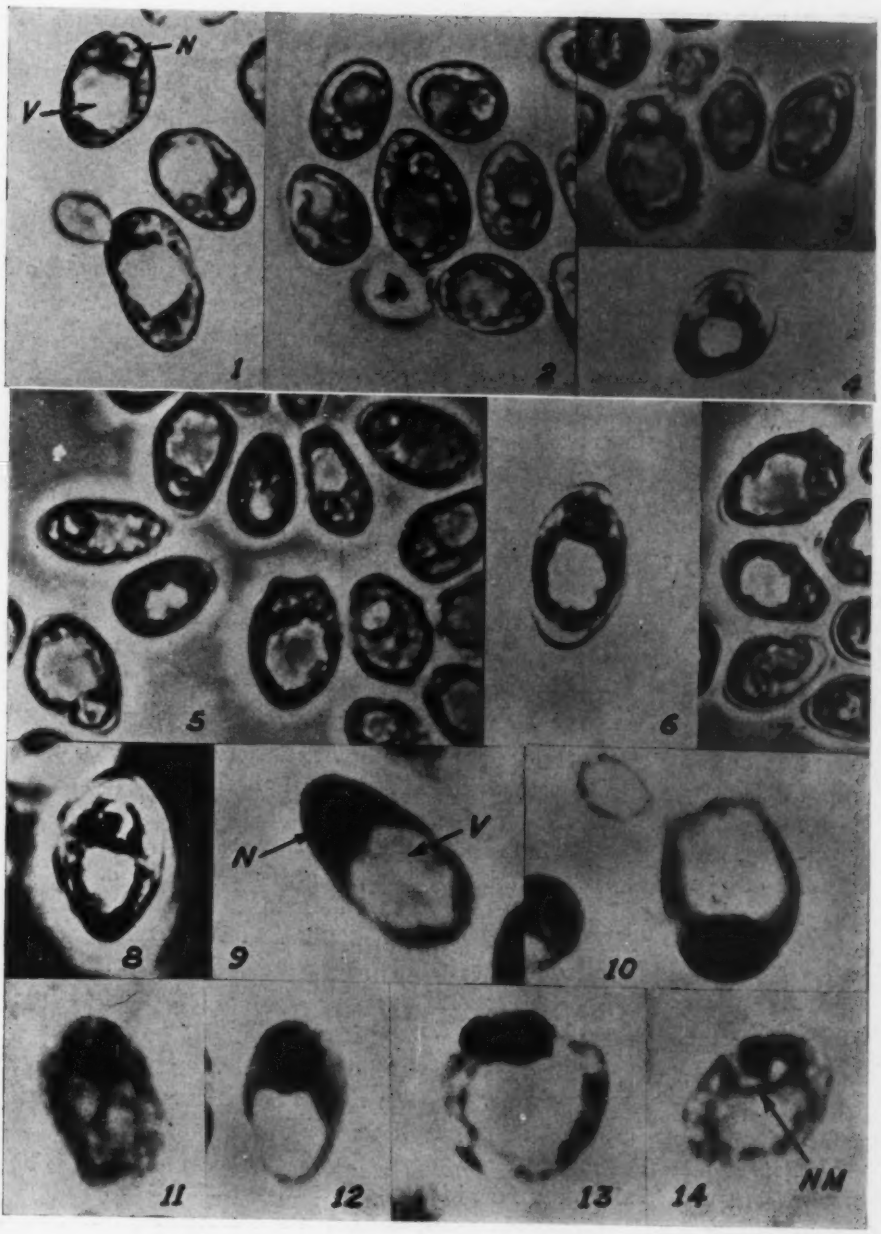
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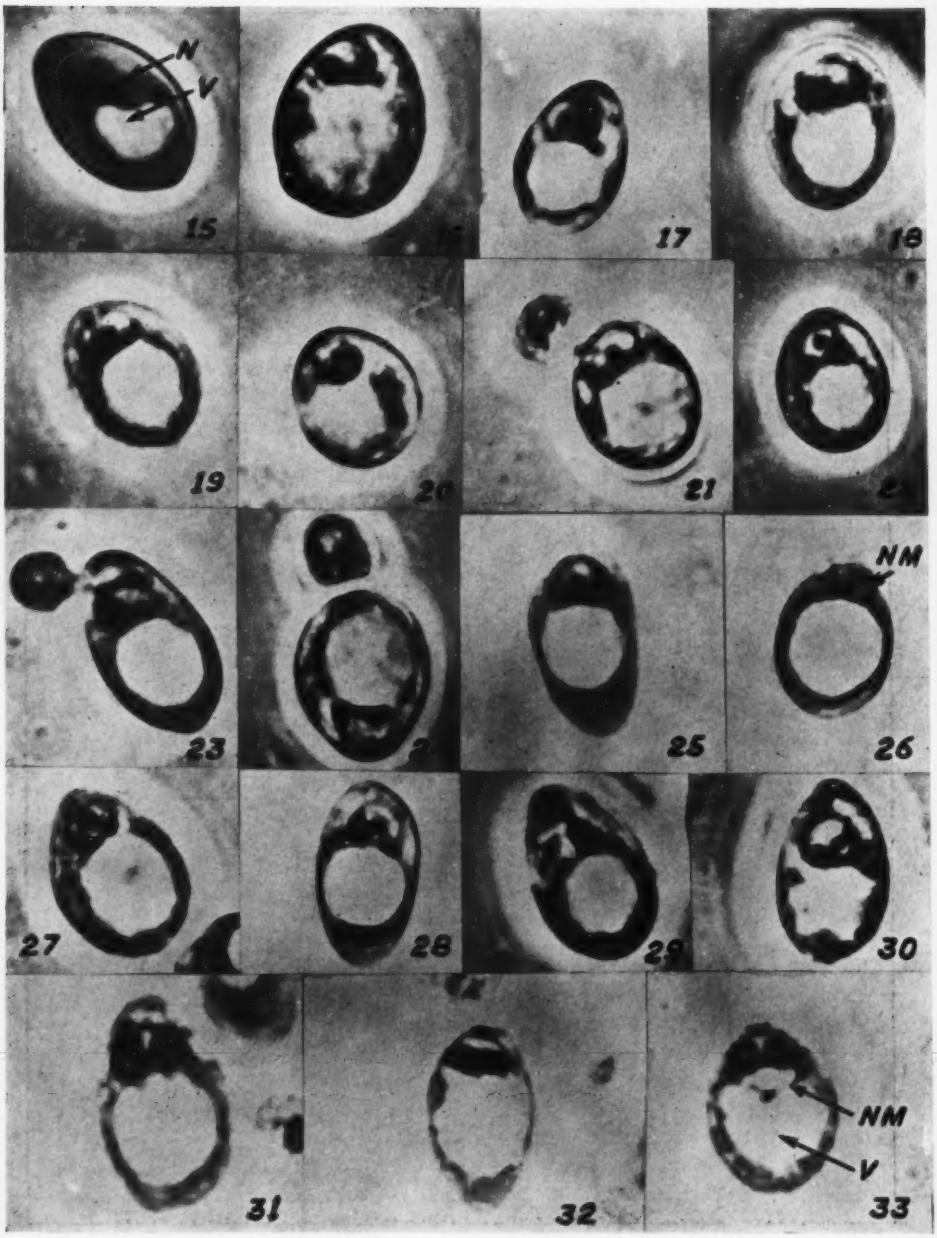
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Explanation of Photomicrographs

Plate III

Photos 1, 3, 4 and 6. Cells fixed in Gram's iodine soln. for 24 hours.

Photos 2, 5, 7 and 8. Cells fixed in iodine-formol-acetic soln. for 24 hours.

Photos 3 and 5. Cells under ordinary illumination after a wash in running water for 2–5 minutes.

Photo 4. A cell under phase contrast after a wash in water for one hour.

Photos 6 and 8. Hydrolysed cells under phase contrast.

Photo 7. Hydrolysed cells under ordinary illumination.

Photos 9–14. Fixation for 24 hours in iodine-formol-acetic, hydrolysis for 9 minutes in N HCl and staining with Heidenhain's hæmatoxylin.

Photo 9. Glycerine mount during the process of destaining.

Photos 10–14. Mounted in Canada balsam.

Photo 10. Nucleus appears homogeneous.

Photo 11. A crescentic unstained area is seen inside the nucleus.

Photo 12. A narrow unstained region separates the two halves stained by hæmatoxylin.

Photo 13. The two stained regions are lying apart.

Photo 14. A distinct nuclear membrane (NM) encloses a pair of stained bodies.

Plate IV

Photos 15–33. Living cells under phase contrast.

Photo 15. The nucleus (N) is homogeneous and projects into the vacuole (V).

Photos 16 and 17. Some areas inside the nucleus appear denser.

Photos 18–21 and 25–29. The nucleus possesses a central grain.

Photo 21. A budding cell with the nucleus in its usual position near the junction of the bud with the mother cell (compare with Photo 24).

Photo 22. Two big grains at the base of the nucleus are lying in the cytoplasm.

Photo 23. The grains disposed around the homogeneous nucleus are in focus.

Photo 24. A budding cell in which the nucleus lies at the opposite end of the cell.

Photos 25–29. The denser regions inside the nuclear membrane give the nucleus a horse-shoe shape.

Photo 26. The nuclear membrane (NM) instead of the denser regions is in focus.

Photos 30 and 31. A region of greater optical density projects into the nuclear matrix.

Photos 32 and 33. A pair of darker areas are enclosed by the nuclear membrane (NM).

Range in size of living cells, 4–8 μ × 6–15 μ. Magnification of Photos 1–9, × *ca.* 2,500 ; Photos 10–14, × *ca.* 4,500 ; Photos 15–33, × *ca.* 2,500.

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(*Vol. XLIII, No. 6, Section B*)

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